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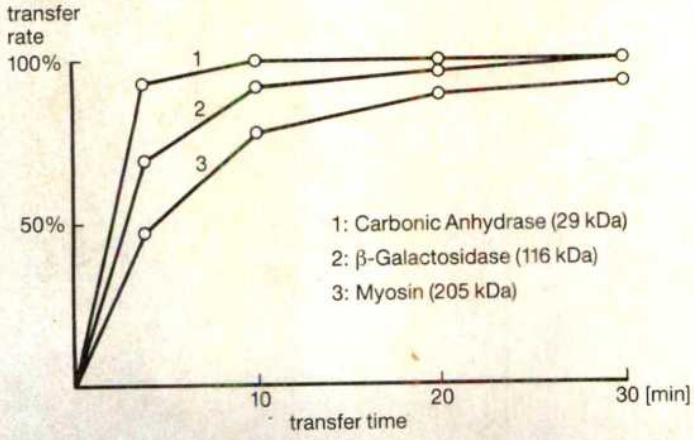
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NESTED SUPERNOVAE

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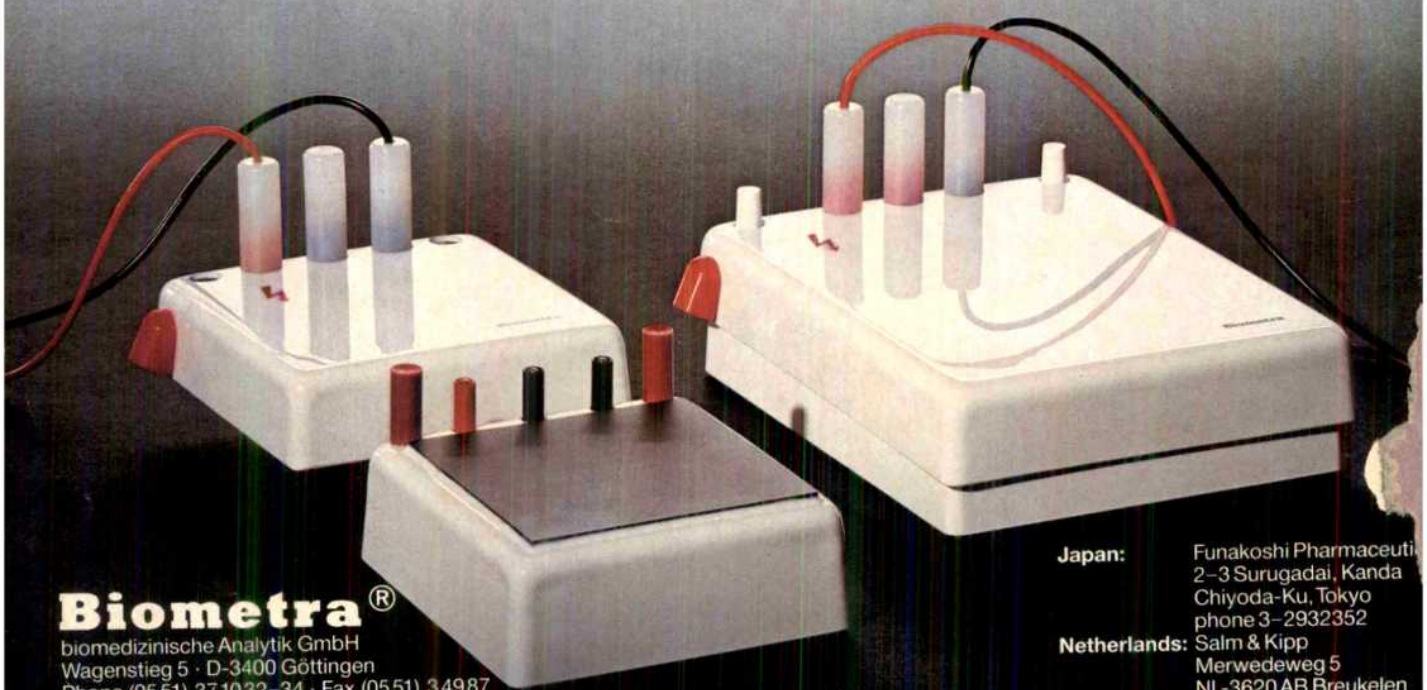
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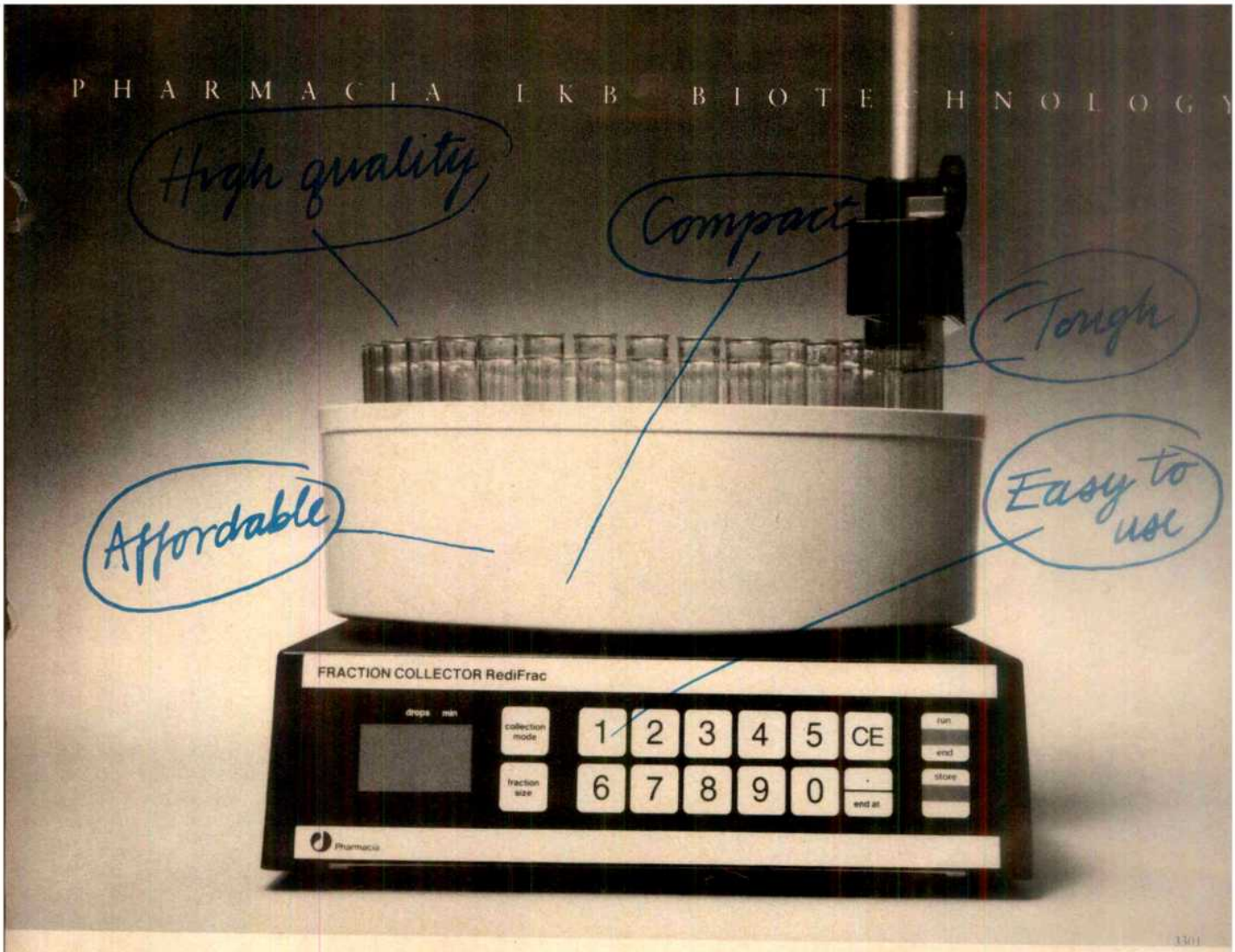
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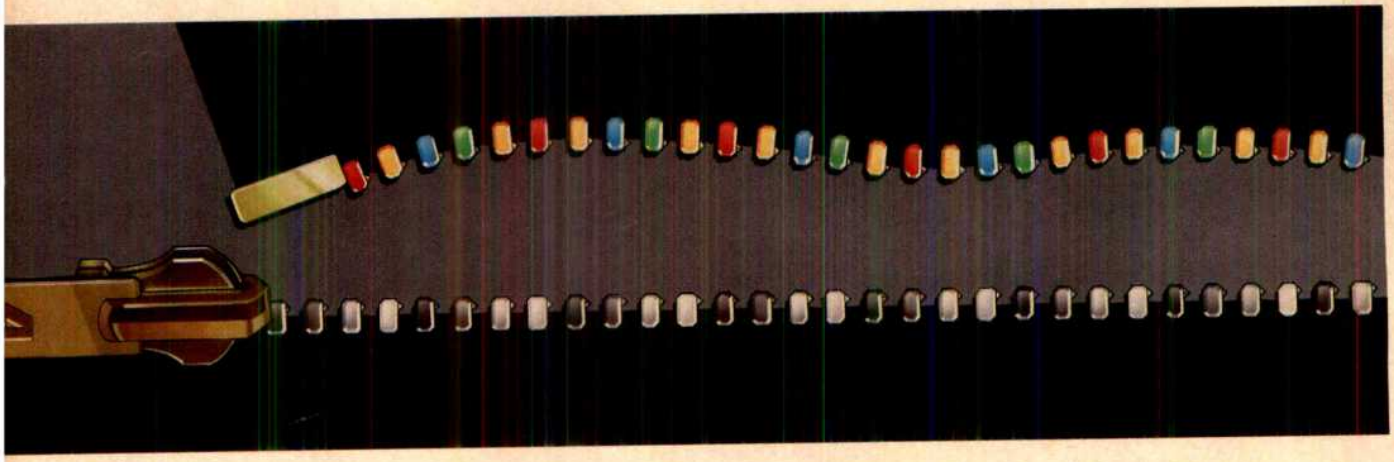
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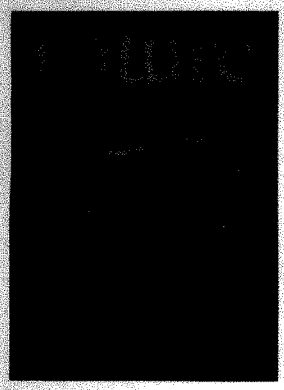
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Filaments within the supernova remnant Puppis A have diverse chemistry, composed primarily of nitrogen (shown red), oxygen (blue), and sulphur (green). These elements, synthesized inside massive stars, may be debris from a second supernova which exploded nearby. See page 48.

THIS WEEK

Seeing the unseen

Can Earth-like planets orbiting other stars be detected? Aperture synthesis could be used to image distant planetary systems at submillimetre wavelengths, page 51. Submillimetre observations are already bringing about a revolution in our view of the Universe, page 15.

Vaccine for seals

Seals can be protected from phocine distemper by vaccination with certain inactivated canine distemper virus vaccines. See page 21.

Molecular chaperones

The *Escherichia coli* heat-shock proteins GroEL and GroES are necessary for the assembly of prokaryotic Rubisco synthesized from a gene introduced into the bacterium, confirming the idea of 'chaperonins' as a general device for assisting the assembly of complex molecules, page 44.

Venusian volcanism

Venus's clouds of sulphuric acid, constantly replenished by volcanic eruptions and depleted by reactions with the planet's surface, will disappear in about two million years if the volcanism moderates. The geochemically estimated rate of volcanism of 1 km^3 per year, lower than two geophysical estimates, can just sustain the clouds, page 55.

Eye of the tiger beetle

A species of tiger beetle uses pointillistic mixing of colours (as in impressionist painting) to produce a cryptic colour pattern, page 72.

Anniversaries for 1989



Mary Evans

The Natural History of Selborne by Gilbert White (here shown aggravating the local fauna) was first published 200 years ago. An anniversarial cornucopia is to be found on page 29.

Modulating behaviour

This week's Review Article considers the part played by neuromodulation in selecting and coordinating coherent behaviours, which can be investigated at the cellular level in arthropod nervous systems, page 33.

Mass extinctions

Substantial changes in carbon partitioning among sedimentary reservoirs accompanied the mass extinctions that mark the start and end of the Mesozoic era (250 and 66 million years ago), but each event has a distinctive geochemical and isotope signature, pages 39, 61, 64 and News and Views.

Moving out

Female social mammals which disperse from their natal groups often do so to avoid interbreeding with their fathers or other close relatives, page 70.

Guide to Authors

See page 96.

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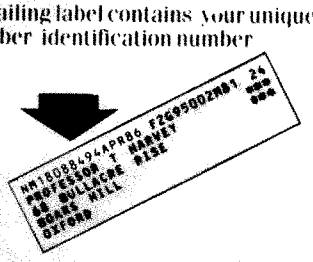
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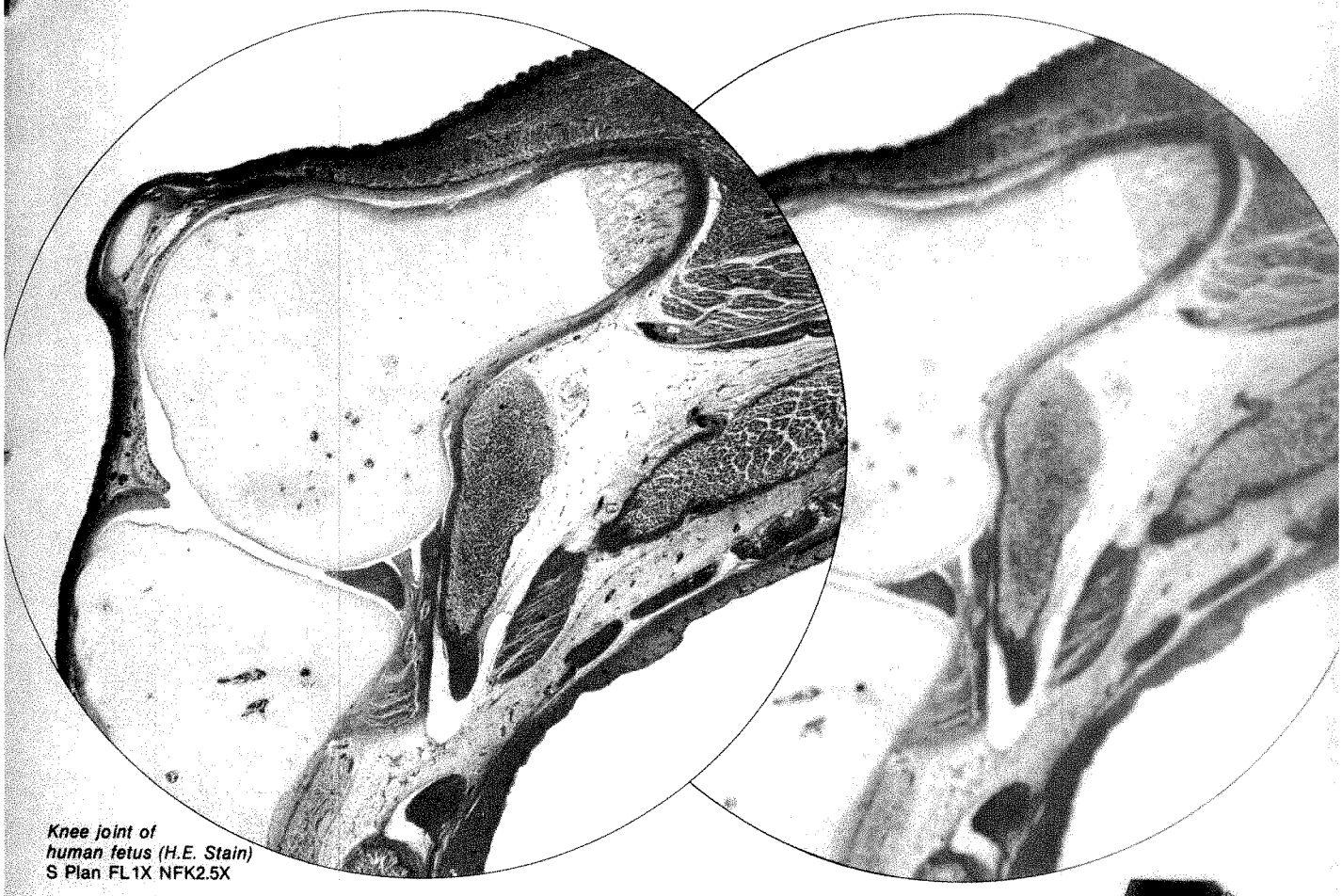
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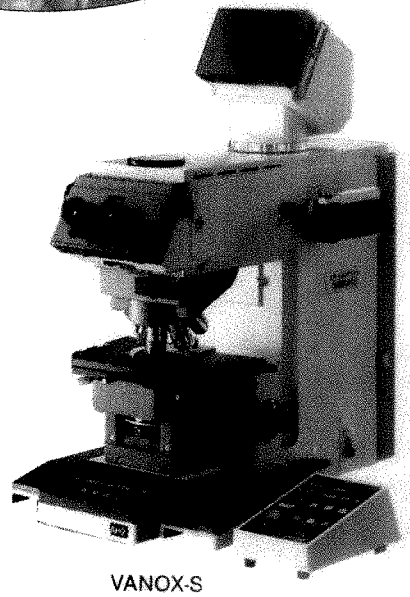
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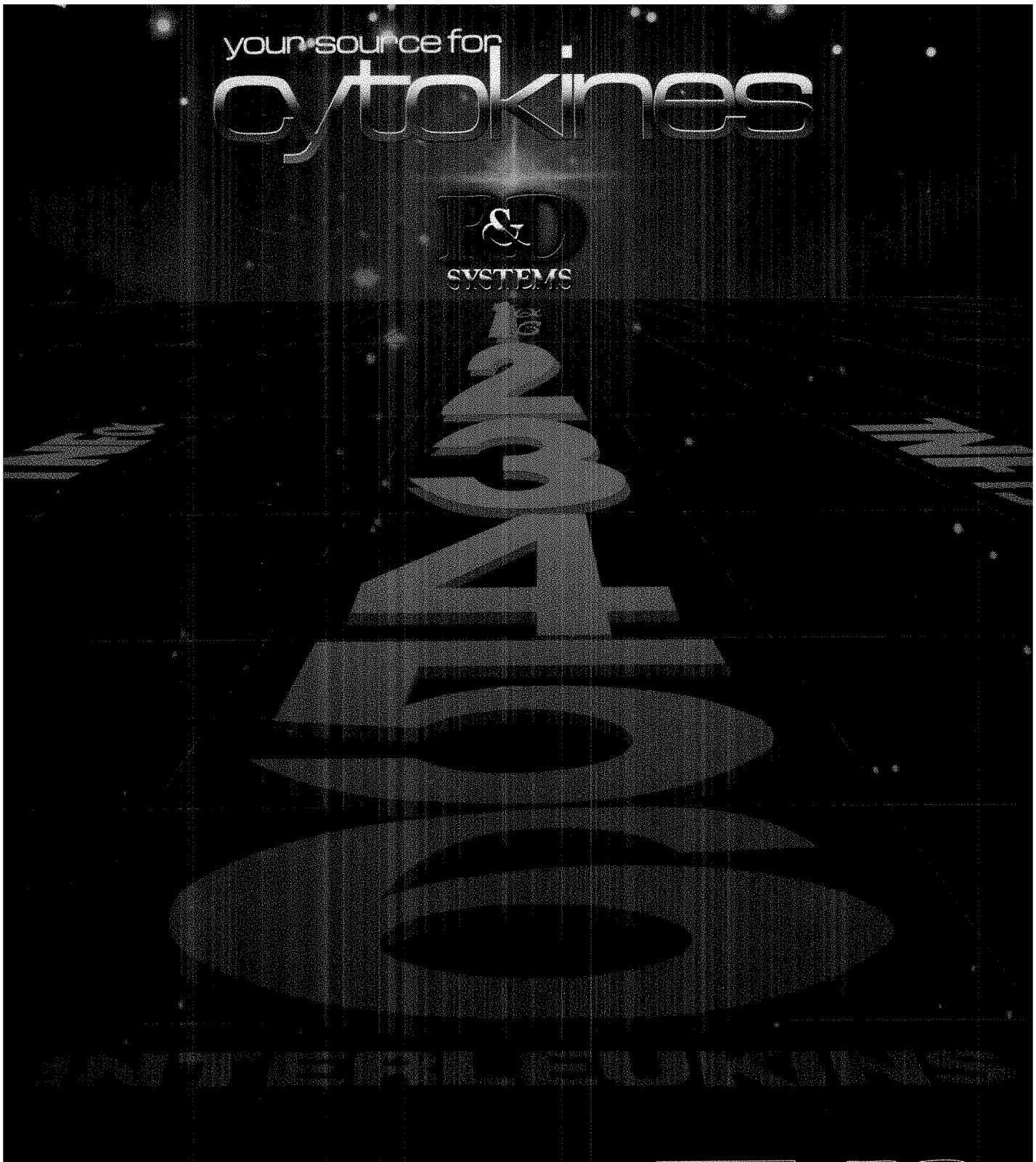


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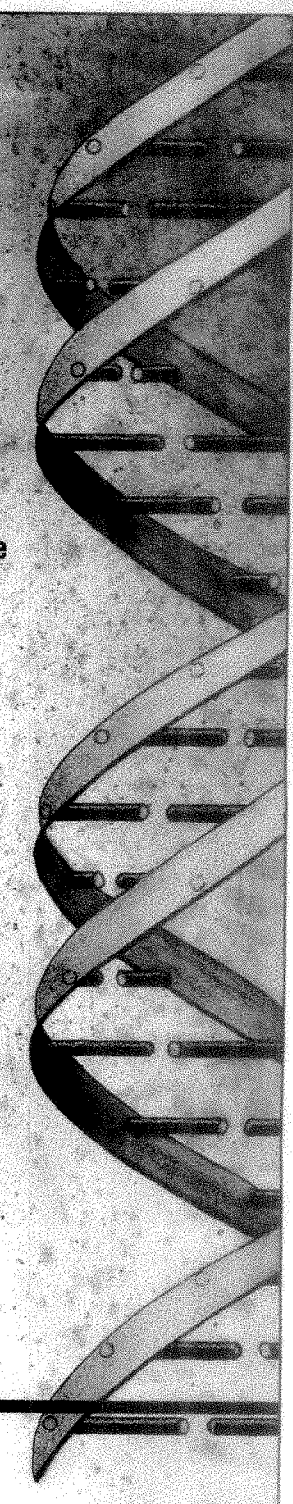
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Monday PM
The Lynen Lecture
I. Neher and B. Sakmann

Monday PM
Molecular Biology of Ion Channels
W.A. Catterall, D.M. Fambrough, Y.N. Jan

Monday PM
Receptor-Based Diagnostics
S.J. Enna, C.D. Johnson, J. Ramachandran

Tuesday AM
The Distinguished Service Award
W. Gilbert

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Regulation of Gene Expression
M.G. Rosenfeld, R.H. Scheller

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Design of Receptor Ligand Pharmacophors
M.B. Bolger, A.D. Strosberg, H.I. Yamamura

Tuesday PM
Cell Recognition and Development
J. Bixby, U.J. McMahan, M. Schachner

Tuesday PM
Nerve Growth Regeneration and Transplantation
A.J. Aguayo, F.H. Gage, F.F. Hefti, J.R. Sladek

Wednesday AM
Molecular Basis of Memory and Learning
D. Alkon, W. Gispen, P. Andersen

Thursday AM
Special Achievement Award
Osamu Hayaishi

Sorting and Localization of Proteins
G.A. Banker, R.B. Kelly, R.L. Rotundo

Thursday AM
Neuropeptides
F.E. Bloom, L.L. Iversen, S.J. Watson

Thursday PM
Second Messengers
K.J. Catt, R. Llinas, H. Rasmussen

Thursday PM
Sensory Systems and Transduction
A.J. Hudspeth, S.H. Snyder, C. Zuker

Friday AM
Molecular Aspects of Neurological Diseases
H.F. Epstein, J.B. Martin, D.L. Price, D.S. Gerhard

THE SECOND ANNUAL NEURAL SYSTEMS SYMPOSIUM: COMPUTER NEURAL NETWORKS: A CRITICAL OVERVIEW

Tuesday
Pattern Recognition as Motivating Neural System Research
David Bendel Hertz

Neural Self Organization
Stephen Grossberg

A Neural Network Architecture For Preattentive Vision
Ennio Mingolla

Adaptive Hand-Eye Coordination
Michael Kuperstein

Optical Realization of Pattern Recognition
Dana Anderson

Stochastic Neural Net and Optical Learning Machines
Nabit Farhat

Neural Networks for Speech Recognition
Richard Lippmann

Wednesday
Instantiation Schemes for Pattern Recognition
Yi-Tzu C. Chien

Optical Realizations of Bidirectional Associative Memories
B. Keith Jenkins

Mapping Multi-Layered Neural Networks for Pattern Recognition Onto Multi-Processors
Genevieve Cerf

Available Recognition Schema
Leon Cooper

Future Directions For Neural Networks
Luis Glaser

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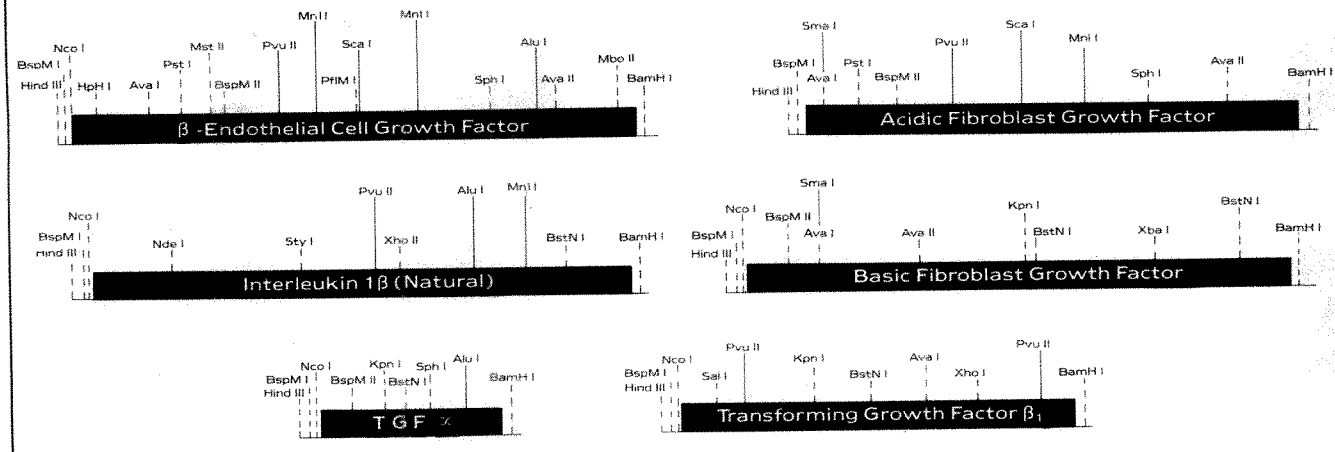
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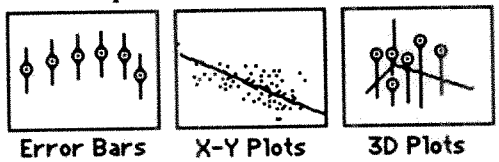
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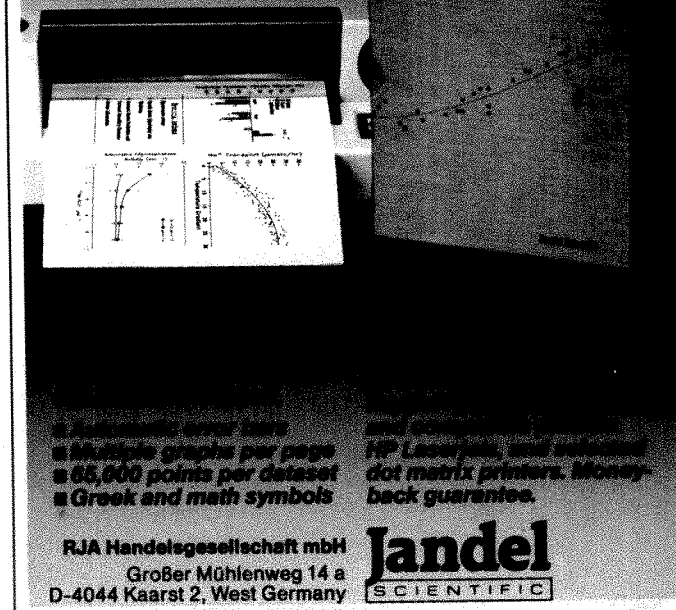
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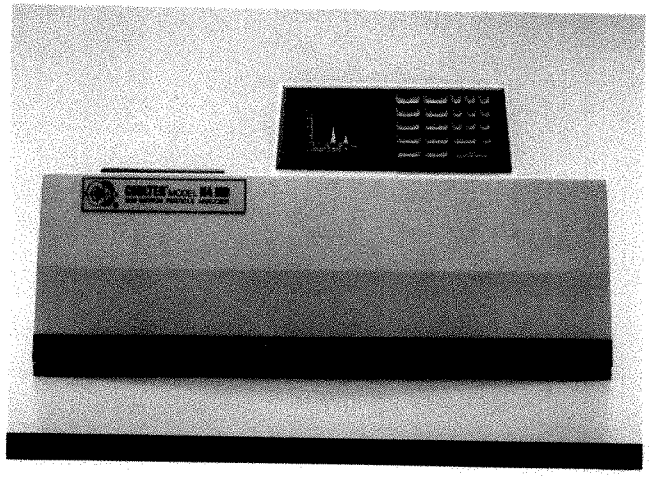
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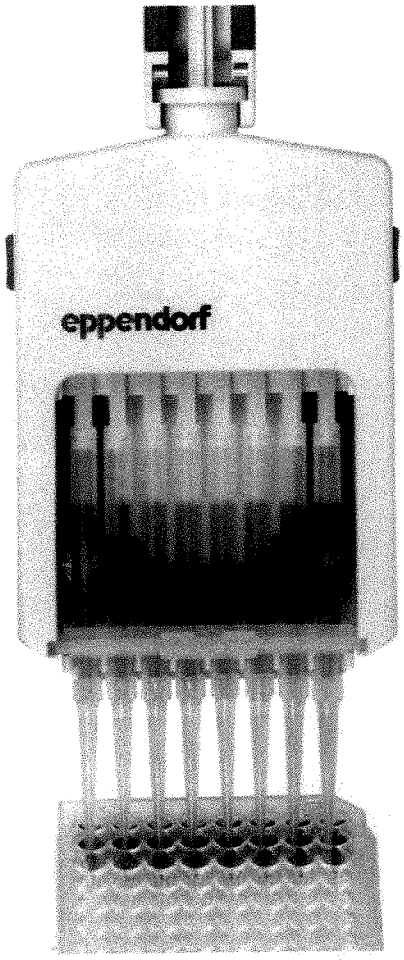
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nature

NATURE VOL. 337 5 JANUARY 1989

Nature's East–West exchange

In the knowledge that the scientific enterprise is genuinely international and in the belief that Mr Mikhail Gorbachev's speech to the United Nations on 3 December offers us all the promise of a different world, Nature *makes the following announcement to and solicitation of its readers.*

WHILE on the face of things there may seem little that the scientific community can do, except to keep on working, to assist or cement the recent transformation of East–West relations, that is not strictly the case.

First, there is ample evidence that the huge Soviet research community, hampered as it is by bureaucracy and shortages of equipment, would benefit from closer relations with the outside world. Individuals might thereby be helped to be more creative. Research laboratories and institutes could well be more productive and effective if their inevitable isolation were abated even modestly. There can be few in the West who would not acknowledge that these benefits must be mutual.

It is also plain that Soviet science has much to offer, even in these competitive times, to people working in the relatively well-equipped laboratories of the West. Those benefits would also be mutual.

In the past few years, public authorities in the Soviet Union and elsewhere have recognized the urgency of these needs. Opportunities for Soviet scientists to travel to the West have been enormously increased, and at the same time liberalized. Travel in the opposite direction has similarly been enlarged. Research collaborations have multiplied, if less quickly. But it is also clear that there is a long way to go before relations between the Soviet research community and those elsewhere are as intimate and constructive as they might be.

Nature therefore intends to take two practical steps to assist these processes.

- First, we shall do more than has been possible so far to draw attention to important trends in Soviet research, largely for the benefit of readers in the West. Talks with the Academy of Sciences of the USSR, as well as with individual scientists, encourage the belief that there is much that can usefully be done.
- Second, *Nature* will create an informal network, to be known as *Nature*'s East–West Exchange, whose chief objective will be to put professional researchers with mutual interests in touch with one another. *Nature* will function as a post-box only, relying primarily on information voluntarily supplied by readers, but will also use its own network of advisers to identify potential respondents to particular enquiries.

It would be rash to guess how well and how generally this arrangement can be made to work, let alone to estimate its importance in the grander scheme of things. But a scheme for exchanging information and easily transportable materials which is entirely independent of government agencies and established academies may have the special value of enabling informal and, with luck, speedy responses to enquiries.

None of this should be taken as diminishing the importance of the bilateral exchange schemes at present operated by national academies and other agencies, or the arrangements for collaborative research involving Soviet scientists and their counterparts elsewhere. It is also a fact that in some fields, high-energy physics or plasma physics for example, Soviet collaboration with others has always been close. An informal private scheme for the exchange of information may nevertheless usefully complement arrangements already in place.

Readers wishing to participate should send in writing their names, addresses, telephone and telefax numbers to one of the editorial offices in London, Munich, Tokyo or Washington. Readers are assured that this information will not be used for any other purpose.

Readers should indicate their interest in the scheme, which may take one or more of the following forms:

- A request for information or assistance, which should be as specific as possible.
- A statement of particular information and assistance that can be conveniently provided, and which is unlikely to be readily available in the East (or West), which should also be specified as fully as possible.
- An offer to help in identifying sources of information and assistance in particular fields of research, which should also be specified with some precision. ("High-energy physics" or "molecular genetics" may invite more requests for help than respondents would wish.)

It is not intended that the scope of the exchange should be limited, but it is expected that enquiries will at the outset be requests for information, perhaps for software developed specifically for basic research, but physical samples (say, DNA probes) may also be suitable for exchange. Respondents should be aware that it is for them, not *Nature*, to comply with whatever national legislation may be in force.

Particular care will be taken to ensure that Soviet scientists at Soviet universities and less well-known research institutes will be able to collaborate.

No charges should ordinarily be made for information or materials supplied under this exchange, although there may be circumstances when two participants agree that costs arising should be paid for.

Nature will publish reports on progress of the scheme at intervals of three months.

A list of people who have endorsed the general objectives of this scheme will be published next week. □

Europe in trouble

The impending trade dispute with the United States will confirm fears about the new Europe.

THE European Community seems well on the way to being an obstruction to international amity. That is the chief lesson that will be learned from the foolish dispute with the United States over the import of beef which was institutionalized last Sunday, the first day of the New Year. The most immediate consequence will be to sour the atmosphere in which some kind of compromise must be hammered out on the future of the international agreements that make the world's free trade an engine of beneficent economic change; after the collapse last December of the Montreal negotiations of an extended basis for the General Agreement on Tariffs and Trade (GATT), it was agreed that the participants have only until April to settle their differences.

But the more distant yet more serious consequences of the dispute will be to confirm the impression that the new Europe is bent on being a selfish and restrictive member of the international community, more concerned with its own interests than with the well-being of the whole world, which is what the critics of the European Community have always feared.

It is especially discreditable that this year's trade dispute should spring from what is represented as a scientific issue. Two years ago, the European Community took the view that beef cattle nurtured with the help of bovine growth hormones would yield beef that endangers the health of those who eat it. There appears to be no factual basis for this supposition, but every reason to suppose that the truth is that beef from cattle grown in such a way is identical with what might be called natural beef.

Certainly the European Commission's directive which, from 1 January, bans the sale of beef whose growth has been assisted by the administration of growth hormones is innocent of an objective test for telling now-tainted beef from that whose sale is to be allowed. Instead, those wishing to import beef into Europe have to obtain a certificate from suppliers elsewhere that their cattle have not been fed growth hormones to help them grow more quickly. And there seems no doubt that the motives for the new restrictions on the sale of beef grown with the help of hormones are simply the commission's wish to make European agriculture less efficient than it could be, and thus a less embarrassing charge on European budgets. Briefly, Europe has decided to ban the sale of beef grown with the help of growth hormones because it does not wish to pay European farmers even more than it does at present for the accumulation of unsaleable stocks of European beef. In a rational world, of course, the remedy would have been much simpler; Europe would have reformed its irrational policy on agriculture instead.

Response

The immediate response of the United States is understandable. With effect from 1 January, retaliatory tariffs will be applied to certain imports of agricultural produce of European origin.

The losers will be of two kinds — consumers in the United States and farmers in the European Community. But there is worse to come. For the European Community now threatens to retaliate against the retaliation instituted by the United States. There is every likelihood that European agriculture ministers, who have many better things to do, will spend their energies in the coming weeks devising lists of agricultural products from the United States to which further restrictions may be applied. The losers, then, will be European consumers, who will have to pay higher prices for goods to which they have become accustomed. But there is every likelihood that mutual retaliati escalate, causing people on both sides of the Atlan become impoverished and creating a climate in which sensible agreements on the future pattern of free trade cannot be reached.

The prospect of a trade war in agricultural produce between Europe and the United States is, sadly, not the only ominous sign of the way in which modern Europe is tending. In the past few months, the European Commission has responded to the pleadings of European manufacturers by deciding unilaterally that Japanese exports of copying machines and electronic printers are too cheap, and has slapped restrictions on them as well. Japan has rightly decided to appeal against these arbitrary decisions, as GATT procedures allow, but that is a time-consuming and expensive business whose outcome must be uncertain.

Before that corrosive issue can be settled, there will no doubt be other European manufacturers pleading successfully for protection from imports from elsewhere which they consider are too cheap, meaning simply that the prices at which goods are offered for sale are inconveniently and unprofitably low. It may not be long before the manufacturers of civil aircraft, or computing machinery, lodge successful claims that they, too, should be protected from competition from elsewhere. While, in the short run, European manufacturers may enjoy the benefits of markets in which they are the sole suppliers, Europe as a whole will lose both the benefits of competitive prices and of underlying advanced technology.

Gloomy outlook

The outlook is all the more gloomy because the United States, the most likely target of European protectionism, is now equipped with domestic trade legislation that makes retaliation almost a statutory requirement. While the Reagan administration has steadfastly, over eight years, resisted attempts by the US Congress to protect particular industries from competition from elsewhere, it was forced last year to concede a provision that industries with cause to believe that their competitiveness in export markets is thwarted by foreign restrictions should have a right to demand retaliation. Already there is an awesome queue of claimants. It will be especially difficult for a new administration, needing to plead with Congress on the federal deficit, to keep these domestic demands at bay. In short, there is a serious prospect that the attempt to negotiate a new basis for an extension of GATT's terms of reference will be aborted by a trans-Atlantic trade war extending well beyond the field of agriculture.

If that is what happens in the months ahead, Europe will have to bear the chief responsibility. But, sadly, Europe is not a single entity, but rather a collection of twelve governments with different interests, most of which are narrow interests. Decisions made in Brussels by the European Commission ultimately reflect the way in which particular governments are able successfully to plead their own causes. That is the reason why the common market fully specified by the Treaty of Rome has not yet come about — and why there remain serious dangers that the creation of the single market now planned for 1992 may itself be aborted by strategems not yet conceived. The same narrow view of European affairs offers an explanation as to why it has been so difficult to move towards a rationalization of the agricultural policies that are Europe's self-inflicted burden.

The next few years unfortunately offer narrow-minded European governments too much opportunity for insisting that further moves towards internal free trade must be accompanied by economic protection from outside competition. The ironical result of that will be a Europe which will be self-contained and inward-looking — one in which Europe's founding fathers would prefer not to live. □

International expedition studies Arctic ozone

- Differences in Arctic and Antarctic depletions
- Specially designed aircraft to be used

Washington

An international expedition to study stratospheric ozone concentrations in the Arctic begins this week in Stavanger, Norway. Led by the United States, the six-week expedition will employ balloons, satellites, high-altitude aircraft and an international data network to obtain a clearer picture of how Arctic ozone concentrations are affected by the unique conditions at the pole.

Arctic stratospheric ozone concentration exhibits some seasonal variation, but nothing like the severe declines observed over Antarctica. Nevertheless there are indications that similar processes are at work in the Arctic stratosphere, leading to a measurable depletion of ozone. More puzzling, according to Daniel Albritton of the National Oceanic and Atmospheric Administration (NOAA) Aeronomy Laboratory in Boulder, Colorado, is a discrepancy between observed ozone depletion and predicted levels based on models that account for Antarctic depletions, suggesting that some other mechanism is at work in the Arctic.

Two specially designed aircraft will be used to collect data on gas concentrations in the stratosphere. A National Aeronautics and Space Administration (NASA) ER-2 aircraft, a modified U2 reconnaissance aircraft, will carry instruments as high as 20 km for seven-hour flights up to 80 degrees North. A DC-8 will operate during the same period, its greater range allowing it to survey the polar vortex. NASA's Robert Watson, chief program officer for the expedition, says that ozone measurements from the Nimbus 7 satellite will help to develop flight plans for the aircraft.

Of particular interest is how chlorine compounds — primarily from chlorofluorocarbons — react with ice crystals in polar stratospheric clouds to form free chlorine which destroys ozone. Because the Arctic is on average warmer than the Antarctic, there are far fewer of these ice-containing clouds. Albritton says this may partly explain why the decrease in stratospheric ozone is not nearly as large as in the south. Albritton is hoping for a cold polar winter so there will lots of polar stratospheric clouds to test the ice crystal hypothesis.

NASA, NOAA and the National Science Foundation together are contributing some $10 million to the expedition. Additional support, comes from Norway, the United Kingdom, West Germany and the Soviet Union. **Joseph Palca**

Computer smugglers too quick

Washington

Last week's arrest in Miami of a Dutch man accused of attempting to smuggle a Vax 8800 computer to Bulgaria highlights the difficulties the United States is experiencing in stopping advanced computer technology from reaching eastern-bloc countries. The difficulties will get worse, according to a new report* on export controls and computer technology released this week by the US National Research Council, as computers become ever smaller and more powerful and international computer networks flourish.

The nations of the Coordinating Committee for Multilateral Export Control (CoCom) have managed to maintain a five-to-ten-year lead in computer technology over the USSR and eastern-bloc countries, claims the report, but far more "focused and flexible" controls will be needed in the future. The biggest problem is that "computer technologies advance much faster than most bureaucratic processes".

A clumsy bureaucracy will end up stifling industry if it does not distinguish between products that can be controlled and those that cannot — such as computers available from foreign sources and computers that are so cheap or small that their trade cannot be effectively halted. "More timely, rapid and expert" reviews are the answer, according to the report.

To help stop the industrializing Asian nations of Singapore, Hong Kong, Taiwan and South Korea from supplying their own increasingly sophisticated hardware to the eastern bloc, the report urges their integration into the CoCom programme.

The rapid development of computer networks has provided an easy conduit for the export of software. The report calls for "further study" to control transborder flow via computer networks.

Software is intrinsically difficult to protect and most should be freely traded, according to the report, with tight controls reserved for military software and for software tools that could help create military software. **Alun Anderson**

**Global Trends in Computer Technology and Their Impact on Export Control*, published by the National Academy Press, 30 December 1988.

Ecologist murdered

Washington

Last week's murder of the prominent Brazilian environmentalist and union organizer, Francisco Mendes Filho, at his home in Xapuri, in Acre province, is expected to increase pressure on the World Bank and Inter-American Development Bank to demand environmental safeguards before they lend money for large-scale development projects. Through his efforts to organize and protect poor rubber-tapping families Mendes, 44, inadvertently found himself allied with ecologists who wanted to stop the destruction of the rain forest for cattle ranching. His murder, allegedly at the hands of local land barons, has provoked an international outcry and a campaign in Brazil to stop foreign loans until justice has been done. Last year, Mendes received the Global 500 award from the United Nations for his efforts to save the rain forest. A.A.

Shuttle complacency

Washington

Although the US space shuttle has successfully resumed flights, the final report of a special National Research Council panel evaluating the redesign of the shuttle's solid rocket booster concludes that there are still problems with the booster.

In addition to specific, minor problems that cropped up on the two recent shuttle flights, the final report, transmitted in a 21 December letter from panel chairman H. Guyford Stever to National Aeronautics and Space Administration head James Fletcher, suggests there is still work to be done. The report warns that budget, time and complacency will push NASA towards a relaxation of quality control. Although the "relaxation" may be justified, the report urges that it be a planned, deliberate process, and calls for continuous system performance checks to establish a data base against which the shuttle's future performance can be judged. J.P.

Animals' legal rights

Washington

A group of animal rights organizations, led by the Animal Legal Defense Fund, has filed suit against three US government agencies. The group is accusing the Department of Agriculture (USDA), the Department of Health and Human Services, and the Office of Management and Budget of "unreasonably delaying" the establishment of regulations to protect animals used in research which were stipulated by the 1985 amendments to the Animal Welfare Act. The amendments would require researchers to anaesthetize animals subjected to pain, and to provide dogs and primates with living environments conducive to their well-being. The USDA says it is still sifting through the roughly 8,000 comments it has received. C.E.

'Virus-proof' computer security system

Boston

CAN computer operating be made safe from invading viruses, worms and determined hackers? Two computer scientists think the answer is "yes", and they have developed a computer security system which they claim is invulnerable to computer viruses such as the one that recently struck networks across the country (see *Nature* **336**, 97 and 301; 10 and 24 November 1988).

The system, designed by Michael Rabin, a computer scientist at Harvard University, and his former student Douglas Tygar, now assistant professor in the School of Computer Science at Carnegie-Mellon University, is an adaptable set of programs designed to regulate access to information on computer systems.

The system, called ITOSS (Integrated Toolkit for Operating System Security), is the culmination of a four-year effort by Rabin and Tygar. Among its features is a battery of built-in 'sentinels' which can be employed either to warn away users from high-security data, or to record every access to sensitive data files without users' knowledge. A 'fingerprinting' algorithm can also alert system operators to any changes made to programs on the system. Included in the program's design are a 'minimum security' protection for all files against 'trojan horse' programs that might come into the system illicitly, hidden in innocuous messages, and a 'maximum security' feature which requires a specified number of system operators each to input commands in order to make any far-reaching changes in the system.

New systems to protect computers are likely to be necessary if computer operators are to gain the upper hand over hackers. Last month, officials at Lawrence Livermore Laboratory gave up their battle with a hacker who had repeatedly infiltrated low-level security files on the laboratory's computer system. Instead, they left an invitation for the hacker to make direct contact with the laboratory's computer security manager. The hacker accepted the invitation and has now promised to stop his attacks. An FBI investigation is nevertheless under way.

Seth Shulman

Increase for West German ocean research

Munich

A SIZEABLE increase in support for ocean and polar research in West Germany was recommended last week by the influential Wissenschaftsrat, an independent science advisory board. The board recommended that an additional DM240 million should be spent over the next few years, with the money to come from both the federal and *Länder* governments.

The largest new investment recommended is for a DM75 million research centre in Hamburg which would bring together researchers from the University of Hamburg, the Max Planck Institute for Meteorology, the German Climate Computing Centre and the Federal Biological Research Establishment at Heligoland.

Research should be concentrated, the board says, on interactions between ocean and atmosphere as well as on the study of structure and function of marine ecosystems. The four institutions involved, which are funded out of as many different purses, would have to agree on how to carry out such an integration. Two new institutes are recommended for the Oldenburg/Wilhelmshaven area, one to study the chemistry and biology of the ocean and the other to study coastal wetlands. Together they would cost DM40 million.

The Wissenschaftsrat also praised the Alfred Wegener Institute for Polar and Ocean Research in its first eight years as a *Grossforschungseinrichtung* (large research establishment). The leader of the Bremen institute, Gotthilf Hempel, wants to broaden its focus to include Arctic as well as Antarctic research and to include even more international cooperation. Hempel is chairman of the Arctic Ocean Sciences Board.

Steven Dickman

£520 million plans for UK science park

London

EVERY British university is trying to broaden its funding base and to forge links with industry. With that aim, a business park with a science centre at its core is planned for a 500-acre site near the University of Bristol. Details of the plans were released on 19 December.

The £520-million venture will include facilities for research, development and manufacturing, with space for 22 companies. These will have access to the 150 researchers based in the science centre who will come from the Universities of Bristol and Bath and the Polytechnic of Bristol. Each institution has a 5 per cent stake, which is paid for in kind, with the provision of researchers and support staff.

A public inquiry must be held before the venture is approved because the site is on land released from environmental protection regulations. But Don Carleton, of the University of Bristol and director of the development company proposing the plans, says the planning authorities support the plans. He expects construction to begin next summer, and the park to create 14,000 new jobs. **Christine McGourty**

Tanks to hold more than fuel

Washington

THE US space shuttle's giant external fuel tank will some day carry scientific experiments of its own, according to a memorandum of agreement signed last week between the National Aeronautics and Space Administration (NASA) and the Space Phoenix Program of the University Corporation for Atmospheric Research.

In his 1988 Commercial Space Initiative, President Ronald Reagan offered the external fuel tanks to anyone who could think of something interesting to do with them (see *Nature* 331, 550;1988). At present, the tanks are unceremoniously dumped into the ocean after providing the propellants to boost the shuttle into orbit.

There is an unpressurized, 5,000-cubic-foot space between the fuel and oxidizer tanks that can hold a pallet containing scientific instruments. According to Peter Riva, a spokesman for the Space Phoenix Program, that space can easily accommodate relatively large, low-technology experiments that can be loaded just before launch. Some experiments now being considered are gas releases into the upper atmosphere and detailed studies of the thermosphere.

Initial plans call for using the external tank during sub-orbital flights, but ultimately the Space Phoenix Program would like to find a way to place the tanks in more permanent, slow-decay orbits. There is no fixed timetable for when the external tanks will be used to carry experiments, but Riva expects the first flight will be sometime in 1991.

Joseph Palca

Neuroscience Center

Boston

THE Harvard-affiliated Massachusetts General Hospital has opened a new $18 million interdisciplinary Neuroscience Center. The centre, made up of 11 separate laboratories and with a staff of more than a hundred scientists, students, fellows and technicians, is financed by a $6.4 million grant from E.R. Squibb and Sons, a $5 million grant from the National Institutes of Health, and by a number of other private sources.

Dr Joseph Martin, professor of neurology at Harvard Medical School, will direct the new centre. Martin stresses the interdisciplinary approach of the centre, saying that chronic neurodegenerative diseases might best be tackled by "understanding their causes at the genetic level" rather than a "shotgun" approach of focusing only on particular symptoms of individual diseases. The centre will house the hospital's neurogenetics laboratory where molecular genetic techniques are being used to study Huntington's disease and Alzheimer's disease.

Seth Shulman

20th-century smugglers use biotechnology

Sydney

In a cloak and dagger operation that could have been lifted from the pages of a spy thriller, a group of wily Australians have combined the traditional trickery of smuggling with high-technology embryo implantation techniques to try to break South Africa's near-monopoly of the $200 million market for mohair wool.

Mohair wool comes from angora goats, the finest of which are bred in South Africa. Exporting them without the permission of the South African National Angora Breeding Society is banned. But in 1986, three Australian farmers raised A$600,000 from Australian goat breeders to try to lay their hands on a herd.

In South Africa they succeeded in buying 269 top quality Angora and Boer (meat) goats. Waiting until Christmas Eve, when they guessed official diligence would be low, they loaded the herd into trucks and set off at night for a 47-hour trip to the border with Zimbabwe. One farmer flew ahead of the convoy in an aeroplane, smoothing out paperwork at more than 20 checkpoints along the way. At the border, customs inspectors were persuaded the trucks contained sheep rather than goats, a move one of the men involved in the trip agreed was "against the spirit of the South African angora industry regulations".

In Zimbabwe, the Australians bred the goats and collected almost 400 embryos which were frozen and flown to the Australian Government offshore quarantine station on the Cocos Islands in the Indian Ocean. The plan was to implant the embryos into surrogate mothers on the Cocos Islands and keep the kids in quarantine. But the original partnership broke up and the frozen embryos were instead sold to Embyrotech, a Western Australian company. Embryotech transported the frozen embryos to New Zealand, which has already built up a herd of 74 Angora kids and 24 Boer kids.

With multiple ovulation and embryo-transfer technology, the number of offspring can be rapidly increased. Once seven years of quarantine are up — essential to ensure the herd is free from the scrapie disease agent — Embryotech has just announced that it will sell all the offspring to Australian farmers, giving them a chance to cash in on the lucrative angora wool market.

Three other angora herds have also been 'imported' to New Zealand from South Africa. In 1987 South Africa introduced legislation that allows the government to confiscate the farm of anyone collaborating in illegal exports. Tania Ewing

A certain uncertainty awaits in Washington's new year

Washington

CELESTIAL mechanics guarantees that in August this year the Voyager space probe will make a historic encounter with the outer planet Neptune. But apart from that, nearly every other scientific endeavour of 1989 is tinged by an uncertainty not felt for 8 years: from 20 January a new man will be in the White House, and although President-elect George Bush is of the same political cloth as his predecessor, there are already indications that new priorities can be expected.

During his campaign for election, Bush promised to raise the status of the White House science adviser to the rank of presidential assistant, but by the year's end when — nearly — all cabinet appointments had been announced there was still no word about who will fill that new position. This may be an indication that science will not in fact receive its promised new importance, but it could just as easily be evidence that Bush has found — as have others before him — that the political and bureaucratic complexities of bringing in a new team can foil the best intentions.

Adding to the uncertainty are a spate of new faces in important congressional appropriations committees. This month, Congress will receive the 1990 budget blueprint prepared by the Reagan administration, but that will certainly be modified by both the Bush administration and Congress before the start of the new fiscal year.

In addition to establishing budget priorities that could spell the end of big science projects such as the Superconducting Super Collider — now scheduled to make Waxahachie, Texas the mecca of the high energy physics world — and the National Aeronautics and Space Administration (NASA)'s Space Station, Congress will also continue to grapple with the issue of scientific misconduct.

The arcane question of the specificity of the Bet-1 reagent may be the central theme of hearings scheduled for early in the year, as a powerful committee led by Representative John Dingell (Democrat, Michigan) resumes its inquiry into a paper appearing in *Cell* co-authored by, among others, Nobel laureate David Baltimore, that has raised complicated issues of how the propriety of scientific data should be investigated. Another committee chaired by Representative Ted Weiss (Democrat, New York), will continue hearings probing conflict-of-interest issues for scientists who work at academic institutions but are supported by private industry.

For NASA, 1988 was a good year, and 1989 promises to be even better. The space shuttle returned to service in September, and a second successful flight followed. Three major scientific missions are scheduled for launch this year; the Magellan Venus radar mapper, the Galileo mission to Jupiter and the Hubble Space Telescope. If, however, the Bush administration decides not to go forward with the space station, NASA will be searching for a new identity, as military and commercial space activities have already been moved elsewhere.

NASA will certainly have a role studying the environment, a topic that will occupy centre stage in 1989. Last summer's drought and heat wave convinced many that greenhouse warming was worth serious study, and finding ways to limit the production of greenhouse gases, including renewed consideration of nuclear power, will be a constant theme.

If new commercial nuclear reactors are still some way off, the Department of Energy must nevertheless finalize plans for a new defence production reactor to restore US capacity to produce tritium and plutonium for nuclear weapons. By the end of 1988, all US reactors capable of producing tritium had been shut down, and a constant stream of newly discovered threats to safe operation of the ageing reactors threatened any early restart.

The National Science Foundation (NSF) is still hoping to see its budget double by 1992, an increase promised by the last administration, but probably out of reach. NSF has moved ahead by providing money for 11 new science and technology centres, and more may be authorized in 1989.

Coping with AIDS will continue to be a major preoccupation of the US Public Health Service and its subordinate National Institutes of Health (NIH) in Bethesda and Centers for Disease Control in Atlanta. Also in Bethesda, the NIH human genome office, led by James Watson, will get into full swing next year, and W. French Anderson, along with colleague Steven Rosenberg, should begin the first-ever experiment involving the introduction of genetically engineered cells into human subjects.

Biomedical research will continue to face stiff challenges from two well-organized lobbies. Animal rights activists will continue to push for restrictions on the use of animals in research, and anti-abortion groups will seek to prohibit the use in research of fetal tissue from induced abortions. But Bush's choice of Louis Sullivan for Secretary of Health and Human Services has at once outraged anti-abortion lobbies and proved that what was past is not necessarily prelude.

Joseph Palca

... agreement with China ... the scene for 1989

New Delhi

IN India, 1988 was seen out with the signing of a new agreement with China on cooperation in science and technology. After having having been cut off from one another for 30 years, scientists of the two countries will now cooperate "on the basis of equality and mutual benefit".

Set alongside increasing technological ties with the Soviet Union — including a promise to aid construction of two nuclear reactors on India's east coast — the agreement may signal a trend for Indian scientists to forge closer ties with colleagues in the communist world. Indo–US cooperation reached a low point in 1988 with disputes over patents and protection of intellectual patent property rights.

The five-year science pact with China is one of the three agreements signed during Prime Minister Rajiv Gandhi's pre-Christmas visit to Beijing. The agreement will provide a framework within which Indian and Chinese institutions can conclude protocols and research contracts. Agriculture and industry will take priority but other forms of technological cooperation may be mutally agreed upon. The agreement also provides for exchange of scientists, documents and journals, and holding of seminars and workshops. From a broader political viewpoint, this agreement and two others on culture and trade are seen as a prelude to an accord on the contentious border issue.

Earlier in 1988, India's success with its first remote-sensing satellite (IRS-1) was offset by the failure of the ASLV-3 launcher and the trouble with communications satellite INSAT 1-C.

India's ambitious space programme hinges on the next flight of ASLV-3 and the May 1989 launching of INSAT-1D. The heavy launch vehicle programme could even be terminated if India decides to concentrate instead on the hyperplane, the Defence Department idea for a space-plane that collects oxygen fuel in flight. Soviet help has been sought.

Indian scientists scored a success with their first low-cost parallel-processing computer and by predicting accurately the behaviour of the monsoon for the first time since the creation of the meteorological department. The government will now launch a three-year project to build a supercomputer.

In the medical field, the birth control vaccine developed by the National Institute of Immunology will enter phase-two trials this year. Another vaccine for sterilizing stray dogs and unproductive cattle has been developed by the same institute and is now for sale.

Materials science, biotechnology and energy are key areas for funding in 1989. The first wave-power plant is being built on the southern coast and a plant is to be set up in Gujarat to extract gas from coal without mining. One nuclear plant will be commissioned in 1989 and 20 more will be built over the next 11 years. The Department of Atomic Energy will have no problems with funds but will find it increasingly difficult to overcome opposition from environmentalists.

On the science policy front, Gandhi has still not acted on his advisers' suggestion for a national commission to integrate science and technology with economic planning. Two controversial decisions on liberalizing seed import and permitting entry of Pepsico in the beverage market have been blamed on the lack of a well-defined technology import policy.

Gandhi has asked scientists to help solve India's problems through technology missions. Telecommunications has provided the first success. India has built its own electronic telephone exchange for cities and the mission intends to supply one exchange every day to rural areas. Over the next few years, backing will be given to missions for drinking water, immunization, cattle improvement and mosquito control. But as yet there is no mission to relieve the sufferings of the thousands of Bhopal gas victims who observed the fourth anniversary of the tragedy on 3 December by burning effigies in front of the Union Carbide factory.

K.S. Jayaraman

Still no hope of a coherent Australian science policy

Sydney

AT the end of 1988, Australian scientists finally found their voices. Dissatisfaction over reduced funding and the constant calls by the government to make science more commercial angered scientists sufficiently for them to reject the label of "wimps" given by Minister of Science Barry Jones and come out of their laboratories and protest.

Hopes had been raised that a coherent long-term science policy would emerge from the reports of a government inter-departmental committee (IDC) set up to look into funding and policy needs and from the Smith committee investigating university research requirements, even though the former contains no members from the scientific community.

But scientists seem likely to be further disappointed. The IDC report was presented to the cabinet in December and, although it has not yet been made public, details of its recommendations are known. The report acknowledges the problems of Australian science: poor funding, increasing student-to-staff ratios and the reduction in graduate students, but warns that any general topping-up of funds might be interpreted as "too little too late . . . The Government (would be seen to be) wavering in its policy directions".

In response to criticism that the administration of science is too fragmented, the report recommends a permanent committee of senior bureaucrats reporting to the Minister of Science to co-ordinate policy and funding. A "distinguished scientist" would also be appointed for a two-year term as Chief Science Adviser to the minister.

The IDC report allocates A$20 million to meet the most urgent needs for laboratory equipment in the year ending June 1989. But the funds cannot be given out until the Smith committee reports in late March 1989. The amount is much less than that requested by scientists. CSIRO alone requested an extra A$75 million for next year.

The government seems relentless. A spokesperson for Senator John Button, the Minister for Industry, Technology and Commerce said "we are trying to force scientists to get funding from the private sector. Historically scientists go away and set their own priorities. They will just have to get used to living in the real world."

But big business may not be interested. Dr Max Whitton, chief of entomology at CSIRO, said "science cannot attract commercial funding if there isn't the viable operating base to attract partners". Professor David Penington, vice chancellor of the University of Melbourne, agrees. "Industry keeps its distance from us rather than developing a vigorous partnership. The idea that this could be resolved through a directive from Canberra is nonsense. Incentives and deregulation are needed."

Australia's scientists are fearful that in 1989 the downward spiral for research funding will continue. The Federation of Australian Scientific and Technological Societies (FASTS) with 60,000 members is determined to have science funding put on the political agenda, particularly with a federal election likely at the end of the year. Professor Frank Larkins, president of FASTS, said: "Fundamental science is the foundation of applied science.

"If the government wants science and technology to pay economic dividends, we need increased funding and a five- to ten-year policy."

Tania Ewing

Too many students, too little cash in German universities

Munich

1988 ended on a note of uncertainty in West Germany with students demonstrating at 40 universities to protest against overcrowding and lack of funds. Overcrowding is at record levels, with at least twice as many students registered as the universities are designed to handle.

A new DM2,100-million government 'emergency programme' announced in mid-December will help, but will make a real difference in only a few faculties. And even if the height of the *Studentenberg* begins to come down in 1989, many professors will continue to be hard-pressed to find time for their research.

University researchers have also been hit by a budget squeeze. The Deutsche Forschungsgemeinschaft (DFG) — the main university research funding body — found it could fund less than half the applications in its priority programmes (*Schwerpunktprogramme*). Overall, DFG turned down more grant applications than ever before, raising worries that young people will forced out of research careers. But DFG takes heart from the better quality of grant proposals.

Researchers at the Max Planck Society are better off. A modest increase in the 1989 budget comes on top of Nobel prizes in 1988 for three of its researchers: Hartmut Michel, Johann Deisenhofer and Robert Huber. Michel has since moved to Texas, but Deisenhofer and Huber remain at Max Planck Institutes.

The Large Research Establishments (*Grossforschungseinrichtungen*, GFE), like the Max Planck Institutes, do not have to worry about the annoyance of too many students. But both associations have promised to cooperate more with the universities, in keeping with the recommendations released in May by the science council (*Wissenschaftsrat*). The council fears an increasing division between universities and research institutes.

In 1989, GFE are likely to place greater emphasis on health research now that Harald zur Hausen of the German Cancer Research Centre is chairman. They have also announced their intention to work more closely with researchers in other European countries.

International cooperation is also the theme for West Germany's burgeoning space programme. The Research and Technology Ministry (BMFT) will increase its investment by 11.8 per cent to more than DM1,300 million in 1989, keeping pace with France and the other members of the European Space Agency. BMFT has stepped up its efforts on its second space laboratory, to be launched by the US shuttle in late 1991. West German groups have also sent payloads on a Chinese rocket and on the Soviet probe to Phobos.

Cooperation may come harder in areas where ethics are involved. Differences began to appear in 1988 between West Germany and other European Community members about research on human embryos and the sequencing of the human genome. Both are emotive issues that will command more attention in 1989.

AIDS was less of a public issue in the second half of 1988, but the liberal policy of the West German government towards AIDS carriers may be in for some changes in 1989 under a new and untried Health Minister, Ursula Lehr, who replaced the veteran Rita Süssmuth in November. Peter Gauweiler, a hard-line conservative, lost the AIDS portfolio in a Bavarian state government reshuffle. On the research front, a modest BMFT programme to establish centres of excellence for AIDS research has yet to bear fruit.

1988 began and ended with nuclear 'scandals' of different kinds. In January, hundreds of mislabelled barrels of nuclear waste turned up unexpectedly at nuclear waste storage sites across West Germany. In December, public trust was dealt another blow when a reactor mishap at the Biblis nuclear plant near Frankfurt was found to have been covered up. Nevertheless, Chancellor Helmut Kohl's centre-right coalition government held fast to its nuclear programme, just as it had after Chernobyl.

1989 will find legislators watching the clock as the Kohl government's second term winds down. The next elections for the Bundestag (parliament) are not due until 1990 but five Landtag (state parliament) elections in 1989 will set the stage for the big campaign. **Steven Dickman**

Return to the good old days of plenty for French researchers

Paris

FRENCH scientists began 1988 facing cutbacks in basic research spending and with instructions to begin paying homage to industry. That, said President Jacques Chirac and science minister Jacques Valade, was the way to bring France into a united European market in 1993, creating the opportunity to compete with Japanese and US technology. But elections in the spring brought a change of government and, with it, hopes of a return to the good old days. Radical changes were made by the incoming socialist government, but it was a long time before a coherent policy was formulated.

Minister for Research and Technology Hubert Curien's first move was to rectify "flagrant deficiencies" in research funding. A $195 million handout was given in June, doctoral bursaries were increased and 150 new research posts created. In the autumn Curien finally gave substance to President François Mitterrand's promises with an extra $484 million increase in his 1989 budget, a 7.5 per cent increase over 1988 and way ahead of inflation.

With the new government settling in during the summer, nothing new was done in the fight against AIDS, even though France continued to rank highest in Europe for the number of AIDS sufferers. Finally, in November, Curien gave researchers $24 million, saying that if it was not enough they would get more, while Health Minister Claude Evin launched a new $8 million AIDS information campaign. 1989 will show whether it succeeds in converting Roman Catholics to the condom, outlawed by the Pope.

The French space industry, major shareholder in the European Space Agency (ESA), continues to boom. Sixteen satellites have been launched in the past 15 months. Ariane 4, the new workhorse heavy-lift launcher, also successfully completed its maiden flight in 1988. Arianespace enters the new year with $2,240 million worth of orders for the launch of 36 satellites. Now all eyes are on Ariane 5, which will be used to send the Hermes shuttle on its way to Europe's Columbus contribution to the US space station, also finally approved in 1988. Work has started at ESA's Kourou launch site to build a new launch complex for Ariane 5, due to fly in 1995, to the chagrin of British space scientists whose HOTOL shuttle is still looking for a sponsor.

Reform, the unlucky buzz-word of the last government, has been replaced by 'evaluate'. The high levels of public spending needed to realize the new government's declaration of research and education as priorities will need careful housekeeping. Curien has introduced a new watch-dog committee to oversee the cost-effectiveness of research. 1989 will also see Jacques Benveniste's research on the 'molecular memory' of water scrutinized as part of a routine, but major 'in-house' review of his laboratory.

Besides 'evaluate', the government's will is to 'co-ordinate'. No major policy decision has been taken without an in-depth report with the emphasis on inter-ministerial co-operation. In France, 1989 will be the year of committees, but only time will tell if their designs produce horses or camels. **Peter Coles**

Japanese science looking to the West and thinking big

Tokyo

IN Japan, 1989 begins with increasing concern that the Recruit stock market scandal, which has already forced the resignation of Finance Minister Kiichi Miyazawa and top leaders in industry, will sweep away others in high places. Further revelations of influence peddling in the political world may mar a year in which Japan wanted to 'internationalize' its image, not least in science and technology.

1989 will be an important year for 'big science'. The Ministry of Education, Science and Culture (MESC) will make available several tens of millions of dollars to begin development of the world's largest helical fusion device at a new institute in Gifu Prefecture. And the Science and Technology Agency will pour similar sums into development of the world's largest synchrotron which is expected to be sited at a "technopolis" in Hyogo Prefecture, near Osaka. Both machines are scheduled to begin operation in the mid 1990s.

Japan will continue its modest but successful space programme with the launch of three satellites. The National Space Development Agency will put up a meteorological satellite (GMS-4) and a marine observation satellite (MOS 1-b). In February, the Institute of Space and Astronautical Science (ISAS) will launch a satellite (EXOS-D) into the 'bow wave' of the magnetosphere above the polar regions of the Earth to observe the formation of aurorae.

Eyes will be on the Space Activities Commission to see if (as is expected) it will grant a request from ISAS to increase the size of its rockets. For the past 20 years, ISAS rockets have been limited to a tail diameter of 141 cm under an agreement between the Science and Technology Agency and MESC. But larger rockets are needed to fulfil ambitious plans to send probes to the planets and beyond.

The first batches of young Western scientists invited under new postdoctoral fellowship schemes will begin arriving in Japan in the next few months. The government fellowships were introduced in the face of US pressure on Japan to open up its research system to Western (in particular US) scientists, and became available shortly after signing of the US–Japan Science and Technology Agreement in Toronto in June. But the schemes are suffering from a lack of applicants and it is uncertain if they can be expanded as planned in fiscal year 1989.

Japan's Human Frontier Science Program, which will be set up as a foundation in Europe in October, may fare better. A 'pilot' programme of five grants offered by the Ministry of International Trade and Industry (MITI) last summer received a flood of applicants from scientists of the summit nations.

The grants, for research on higher-order brain functions or molecular-level recognition and response, are open to scientists in institutions located in three or more summit nations, one of which must be Japan. When the foundation opens in Europe, the grant programme will be expanded and the foundation will also award postdoctoral fellowships and stage international workshops.

It remains to be seen how much control the Japanese government will maintain over the foundation. MITI projects have tended to suffer from inflexibility; much will depend on the make-up and nature of governing bodies to be established for the programme.

Speedy internationalization will be seen in the private sector. In May a consortium of UK, US and Japanese companies headed by Britian's Cable and Wireless and C. Itoh of Japan will begin international telecommunication services in competition with Japan's Kokusai Denshin Denwa (KDD). The consortium will also begin laying a trans-Pacific optical fibre cable to compete with a new KDD cable that will come into operation in March.

Research and development will begin to move abroad with the opening of an institute sponsored by electronics giant NEC at Princeton in May. The institute is expected to set a trend, as is a three-year joint agreement between Hitachi and Texas Instruments to develop 16-megabit dynamic random access memory chips.

The Recruit scandal is thus all the more damaging because it comes at a time when Japan is trying to improve its image overseas. The Recruit saga reveals that 'money politics' still rules Japan and little has improved since the Lockheed scandal of the 1970s which brought down former prime minister Kakue Tanaka. Many questions remain unanswered, particularly regarding the involvement of former prime minister Yasuhiro Nakasone in the affair. And although Prime Minister Noboru Takeshita is now riding high because of the successful passage of a major tax reform bill, he is not immune from attack.

Takeshita's strategy will be to try to put Recruit behind him by launching his pet 'home town' plan for the development of the regions and decentralization of Tokyo. But the popularity of his administration has plummeted and he will have much to do if the party is to choose him to continue as prime minister in the autumn.

David Swinbanks

Glasnost doing *perestroika* in

London

DURING 1988, the Soviet Union had to come to grips with *glasnost*. From the February fire at the Academy of Sciences library in Leningrad, through the posthumous revelations of Valerii Legasov about the Chernobyl disaster, and up to questions as to why, after so much research investment in earthquake prediction, Spitak and Leninakan were unprepared, people in authority have had to accustom themselves to the unfamiliar sensation of being publicly questioned.

In science and technology, passing responsiblity for failures back and forth between the research and production sectors will no longer do. The All-Union Academy of Sciences has completed its replacement of superannuated personnel (and elected Andrei Sakharov to its presidium), but has had its own scandal: the destruction of a major archaeological site for the building of its Crimean rest home. There have been major reassessments of history, many posthumous rehabilitations and 'rediscoveries' of disgraced party leaders, scientists, and scholars.

Yet *perestroika* (restructuring) is running slower than *glasnost*. Self-financing for industry and family leasing for agriculture have yet to show their economic results, and some publicized reforms have failed to materialize. Many managers still seem beset by the 'gigantomania' of the Brezhnev years. The most promising development of the year — an education reform that, it is promised, will at last allow excellence to flourish — was announced only in its closing days.

A major feature of 1988 has been the increase of 'national' demands throughout the 15 constituent republics of the Soviet Union. Some of the demands — such as the development of scientific vocabularies for the 'national' languages based on 'universal' rather than Russian roots, or the establishment of a separate Academy of Sciences for the Russian Federal Republic — should not prove too difficult to satisfy. Nor should some demands of environmentalists — such as the campaign against the chemical works in Erevan. But recent local campaigns in the energy sector require a change in policy.

Opposition to nuclear power has grown and several major projects — including the Minsk nuclear power and district heating station, and the Vitebsk, Chynyryn and Crimean nuclear power stations — have been cancelled or converted to conventional fuel. The Armenian nuclear power plant will be phased out by 1990, and further extension of the Ignalina power station in Lithuania has been cancelled. But these concessions to local opinion still

better than Soviet Union

remain decisions taken in Moscow. The governments of the individual republics still have no say in the development of nuclear power within their territories, and so far only the Lithuanian Supreme Soviet has decided to defy Moscow and withdraw its financial support from nuclear energy.

There is no clear alternative to the Soviet Union's commitment to nuclear power. Large hydroelectric dams are increasingly seen as uneconomic and harmful to the environment, except perhaps single dams on mountainous rivers. But in the mountains of Soviet Central Asia, the local population is alarmed about the seismic hazards of large dams. Proposed solutions such as solar energy in Moldavia or wind farms in Byelorussia seem somewhat utopian.

In the coming year, Soviet energy planners will have to address the difficult problem of restoring confidence in the All-Union energy programme.

Confidence in the space programme also needs to be restored. Space 'achievements' are increasingly viewed by the Soviet public as wasteful attempts to gain international prestige. The partial failure of the Phobos mission did not help to arouse enthusiasm for a manned mission to Mars some time between 2010 and 2020, and a new society to stimulate interest in space among young people has so far failed to take off. The socialist allies feel excluded by the new Soviet commitment to 'international' projects involving France and neutral and non-aligned nations.

Perestroika has set a challenge to the socialist bloc. Reactions range from those of Hungary, where the breakaway Democratic Trade Union of Academic Workers is now a recognized part of the political scene, and Poland, where the Academy of Sciences has just rejected a government report on the environment, to Cuba, which opposes the whole idea of *perestroika*, and East Germany, which claims that reform is unnecessary. In between come Bulgaria, where lysenkoism has only just been finally dispatched with a massive change of personnel in the Academy of Sciences, and Czechoslovakia, where calls for restructuring remain largely on paper.

An encouraging development in 1988 was the holding of international conferences by two new non-governmental organizations based in the socialist bloc — the Hungarian-based International Association of Physics Students and the Bulgarian-based 'Ecoforum'. Both meetings attracted wide support from East and West and were hailed by Soviet 'establishment' participants as a manifestation of the recent trend in 'informal' initiatives.

Vera Rich

Guarded optimism greets increased funds in Britain

London

BRITISH science enters 1989 on an optimistic note, even though 1988 saw no improvement in university finances nor in those of the research councils. The raised spirits in the research community stem from the money for science won from the Treasury by the Department of Education and Science. An extra £95 million is being added to the budget of the research councils for the year 1989–90, an increase of 16 per cent on 1988–89. Specific programmes will absorb much of that money, but, with the remainder, the research councils will be able to support some more research and fund a few more alpha-rated projects. Exactly how the money is to be spent will be announced soon.

Although the award is not extravagant, it is a real increase in science spending and reflects a change in the government's attitude towards science. That change was marked by a speech made to the Royal Society in 1989 by the prime minister, Margaret Thatcher, in which she expressed a commitment to the public funding of basic science.

But research dubbed 'near-market' suffered at the hands of the government in 1988, and the effects of this hit the research councils hard. A review of the work carried out by the Agricultural and Food Research Council (AFRC) identified research valued at £33 million a year as near-market. If the cuts are implemented in 1989, the council estimates that 750 jobs could be at risk.

The Natural Environment Research Council (NERC) was another victim of government policy regarding near-market research. In 1988, the council was forced to cut its support for university research by £2.6 million and to cancel the grants round for October. It announced 130 redundancies to take effect before April 1989, some of which will be compulsory. The council hopes that the worst is now over and that 1989 will be more stable. But the council's new chairman will be fighting to retain not just financial support but the council's identity as the year is likely to bring a merger with the AFRC.

The question of the structure of the research councils resurfaced in 1988 and two committees will report early in 1989 on what form support of the biological sciences should take. The House of Lords committee reviewing support for the biological sciences recommended a merger between the NERC and the AFRC in its interim report in 1988, but in its full report may recommend further evolution towards formation of a council for all the biological sciences which would incoorporate some research of the Science and Engineering Research Council as well as the Medical Research Council. A committee for the Advisory Board for the Research Councils will also give its view in the spring.

Biologists in the academic science community must be looking forward to 1989 with some trepidation. Following the reviews of the Earth sciences, chemistry and physics in universities, which were published in 1988, is the review of the biological sciences. A draft report is now ready; if, like the chemistry and physics reviews, it recommends a concentration of resources in departments of a minimum size, 1989 may witness the beginning of the end of the small science department.

Overseeing the restructuring will be the new Universities Funding Council (UFC), set up in 1988 to take over from the University Grants Committee. The new chairman of the council, Lord Chilver, is embroiled in controversy even before he takes up his post, declaring his view on access to higher education: courses should be available to those who value education highly enough to commit resources to it. His views will find favour in government, which made moves in this direction in 1988 with the publication of a policy document on student loans. Issues such as the repayment of loans and the involvement of commercial banks are still to be resolved, but the government aims to introduce the system in 1990. More protests from students — such as those resulting in clashes between students and police in the streets of London in 1988 — seem likely in 1989.

Dominating the work of the heads of universities in 1988 was the Education Reform Act. On the question of the abolition of tenure, the government emerged the victor — abolition will go ahead — but on the introduction of a system of 'contract funding', the universities' heads emerged victorious. They won a concession from the government that before any conditions are laid down on funds from the UFC, bodies representing the universities will be consulted. But the value of this victory was thrown into question when the Committee of Vice-Chancellors and Principals received from the Department of Education and Science a draft copy of the guidelines to govern the relationship between the government, the UFC and the universities, which described controls the government would have over universities' finances. While the government is encouraging universities to seek a broader base of funding, it seems determined to monitor those finances. Retaining their autonomy will still be high on the agenda of the universities in 1989.

Christine McGourty

History of 'biotechnology'

SIR—Although old uses of the word 'biotechnology' have been noticed[1], no satisfactory lineage has been traced. I should like to draw attention to a source that fulfills three strict criteria: a self-conscious claim to novelty, recognition from others of that novelty and noticeable use of the word.

In 1919, Kark Ereky, the Hungarian agricultural economist and, briefly, minister of food in the counter-revolutionary government, published an 84-page manifesto entitled: *Biotechnologie der Fleisch-, Fett- und Milcherzeugung in landwirtschaflischen Großbetrieb*. Following the precedents of chemical technology and mechanical technology, Ereky coined his new word to cover the interaction of biology with technology, connoting all production by means of biological transformation:

Auf Grund des gleichen Gedankenganges weist der Verfasser alle die Arbeitsvorgänge, bei denen aus den Rohstoffen mit Unterstützung lebender Organismen Konsumartikel erzeugt werden, dem Gebiete der Biotechnologie zu. [original emphasis][2],

which may be translated as: "On the same grounds the author classifies as biotechnology all the lines of work by which products are produced from raw materials with the aid of living organisms".

Ereky's book was well received and its originality recognized. *Die Naturwissenschaften* described the author' goal as a new branch of knowlege that he calls *Biotechnologie*[3].

Thereafter, the word biotechnology appeared in various contexts in the 1920s and early 1930s. Some applications clearly follow from Ereky's formulation. It appears immediately as a heading in *Zeitschrift für Technische Biologie*[4].

Other uses should not, however, be attributed to Ereky. The similiar word *Biotechnik* had come into use at about the same time, and although there is a difference in nuance, even in German the two words could be interchanged[5]. In English translation, the distinction is almost entirely lost. *Biotechnik* therefore spawned the alternative meaning of the English biotechnology. In one of the first English uses, *Nature* entitled a 1933 editorial "Biotechnology"[6]. Dealing with technology and the problem of maintaining the quality of the human species, its title seems to refer to the concept of *Biotechnik* presented by Goldscheid in his social biology of 1911[7].

Since then the word biotechnology has been reformulated many times: in 1947 to describe ergonomics[8], in 1962 as the title for a new journal dealing with biochemical and microbiological engineering[9] and in 1979 to describe the potential of genetic manipulation[10]. The distinction between different usages has often been overlooked because they do share an important connotation: a new biological approach to a great range of industries. This breadth was foreshadowed in the aftermath of the First World War by Ereky's formulation of the concept of biotechnology.

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Primer availability

SIR—The possible applications of the polymerase chain reaction (PCR) (see ref. 1), which amplifies specific DNA sequences during successive cycles of DNA denaturation and extension of specific oligonucleotides (primers) by DNA polymerase, are enormous, both for basic research and for molecular diagnosis of inherited disease. In most laboratories involved in molecular diagnosis of genetic diseases, PCR will gradually replace routine DNA techniques of Southern blotting and hybridization with radioactive probes. A prerequisite to perform PCR, however, is the availability of the specific primers.

Recently, I requested primers for prenatal diagnosis of two common genetic diseases. On both occasions the answer was negative, although the papers describing the primer sequences and their possible applications had already been published. Costs of synthesis and distribution of the primers were probably the main factors hampering their release. If this becomes a general policy, laboratories will each have to face the problem of synthesizing the respective primers for all the different diseases they are studying.

Most companies charge $1,000–$2,000 to synthesize 10 micromoles of a 20–30 base pair oligonucleotide, which is enough to provide 100 laboratories with primer for 1,000 PCRs. With small fees ($15–$20) from each of the laboratories requesting the primers, costs for synthesis and distribution could easily be covered. It might be opportune, for logistic reasons, to centralize primer synthesis and distribution in an organization such as the American Type Culture Collection (ATCC), which has become a central depository and distributing organization for DNA sequences.

The free release of DNA probes to laboratories throughout the world has contributed largely to the exponential increase in our understanding of genetic diseases. For the future, it is essential to guarantee the availability of the necessary PCR primers.

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Soviet refusniks

SIR—We appreciate the increase in the number of exit visas from the Soviet Union granted to Jews, including some long-term refusniks who want to emigrate.

But despite the sizeable increase in the number of visas, a substantial number of long-term refusniks have no foreseeable hope of leaving. Several of them have been waiting for more than 15 years, and some have been told that they will not be allowed to leave until 1992 or 1996.

In most cases, the reason given is that of having access to secrecy. For instance, the Uspensky family has been refused because Igor Uspensky's mother, who is in her late seventies, worked in the Ministry of Agriculture until she retired 12 years ago. She has been told that the reclassification of her secrecy will take place after 1996 (when she will be 84 years old). This not only prevents her from leaving but also her son, daughter-in-law and grandsons. If they were not so tragic, one could put such classifications in the realms of the 'Theatre of the absurd'.

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Biblical criticism

SIR—The recent note (*Nature* **335**, 203; 1988) on the history of the survival of the Aleppo Codex describes the codex as the Hebrew Pentateuch, the first five books of the Old Testament. But the portion reproduced is of a passage from the Book of Judges (9:57 to 11:2). It includes the first part of the story of Jephthah — his exclusion from his paternal inheritance by his step-brethren because he was "born of another woman".

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● The Aleppo Codex originally contained all the books which now constitute the Old Testament; about one-third of the text has been damaged or destroyed. Editor, *Nature*

Prospect for a new year

The temptation to guess what lies even immediately ahead must be resisted, as the prospects for fundamental physics show all too plainly.

PRETENDING to predict the future of discovery is not merely foolish, but a contradiction in terms. Discoveries are, of their nature, unexpected to some degree. What follows should therefore kindly be regarded not as a set of predictions but as a list of wishes. The exercise is worthwhile not for its content, but because it reflects the way that at any time there is a sense of expectation attached to some fields of research, and of dormancy to others. Perhaps a later Thomas Kuhn will think it worthwhile to compare these expectations with the realities that emerge.

Expectations are most vividly engendered by big machines, most of which are built to timetables advertised in advance. The year ahead should see the commissioning of two machines of great importance — the new electron-positron collider (LEP) at the European high-energy physics laboratory (CERN) at Geneva and the Hubble Telescope, which should at last be launched when the US shuttle is back in service and has room to spare. Curiously, neither machine has done as much to lift the spirits of those concerned as might have been expected.

The difficulty at CERN is that, this time, there is no obviously spectacular prize to win, such as the predicted discovery of the mesons that mediate the electro-weak force, and whose masses were predicted to be within the range of collisions between protons and antiprotons in CERN's super-synchrotron. Instead, as if discomfitted that the top quark has not turned up in the same range of energy, and as uncertain as ever where the Higgs particle may lie, people are now hoping that 'new physics' will provide LEP's fun and excitement. Nobody belittles the importance of being able to measure the electro-weak bosons in plenty, but the hazard of new physics is the difficulty of telling the difference between the real thing and spurious data.

The disappointments of the Hubble Telescope are different: frustration and continuing uncertainty. The fact that an instrument which, if it works half as well as planned, is likely to do more to change our view of the Universe than all this century's optical instruments put together should still be sitting on the ground at Los Angeles after three years, waiting for a firm launch date, may help researchers appreciate their properly modest place in the wider scheme of things, but it speaks wonders for the endurance of the human spirit in the face of frustration that the Space Telescope Science Institute and its principal investigators remain cheerful.

Some say the delay may have helped ensure success. The guide stars on which accurate pointing of the telescope will rely have been more accurately and comprehensively measured, while ground access has got rid of an optical misalignment together with a huge amount of unwanted dust. But the telescope has been frozen in design during a period when telescope designers have been more innovative than ever. There is also some concern that the modified shuttle may not be able to put the telescope into a safe orbit.

Either of these machines might help throw light on the great cosmological issues — but not in their first year. Appropriately, the Hubble Telescope should eventually provide a reliable distance scale for the Universe, and thus decide what Hubble's constant is, but everybody understands what painstaking work that must be. Even the issue of what quasars are will take time, though opinion does seem to be drifting towards the view that the nuclei of most kinds of active galaxies embody black holes; sadly, less attention is being given to questions such as the lifetime of the active phase and the related question of whether an active phase is an evolutionary stage of most, perhaps all, galaxies.

Expectations of the contributions likely to be made by particle physics to cosmological understanding are now less uniform than a few years ago, when the Big Bang seemed to many to be nature's practical demonstration that the particles of the known hierarchy (nobody calls them "fundamental" any more) are related to each other by the progressive breaking of a natural symmetry in a mass of expanding and thus cooling gas. Part of the trouble is psychological; no sooner had the particle theorists sketched in the details of the Big Bang in the first few seconds and minutes (with observationally important predictions of deuterium abundance and spatial uniformity in the early Universe) than, it seemed to cosmologists, they fell under the spell of string theory, which is a way of accounting for the differences between particles by means of dynamical variables in dimensions (most commonly, there are six of them) which are hidden from observation, or are "collapsed".

In principle, of course, particle physicists may justly say that the attractiveness of string theory does not imply an abandonment of the exciting vision of how particles run down the heirarchy in the Big Bang, transforming from one species to another in the process, but merely a quest for a more natural explanation of the relationships between particles.

But they have nevertheless damaged the simple view that particle physics and cosmology would come together through the Big Bang in at least two ways. First, the mere suggestion that there may be unobservable dimensions is an unsettling reminder of other conundrums, the quantization of gravity, for example. (The question whether the hidden dimensions may embody the hidden variables for which Einstein fought is too little discussed.)

Second, if the dimensionality of space–time is to have a place on the agenda of discussion, should not attention also be paid to the arguments in the second half of Stephen Hawking's recent book *A Brief History of Time* that the Big Bang is an illusion brought about by occupancy of what is bound to seem a Universe in a corner of a larger structure?

It would be good if there were some progress in dealing with this muddle in the year ahead, because people's instincts are sound and the thinking will have to be done at some stage. On a less abstract but equally teasing plane, it does seem certain that 1989 will be the year of Berry's phase — the notion that for any mechanical system represented by a complex field (such as a common-or-garden electronic wave function), whose phase is supposed to have no physical significance, each characteristic of the system can be used to define a phase in such a way that supposedly identical states have different phases, which may be measured as, or inferred to be, different. Abrahamov and Bohm, independently of M.J.Berry, have shown that the supposedly insignificant complex phase of Maxwell's electromagnetic potential is measurable. Is this another way of importing hidden variables? People are now so busily generalizing Berry's argument that we should know what it means before the year is out. The Nobel physics committee would be well advised to plump for this trio before it is denied the topic by an embarassment of choice. After all, the looked-for explanation for high-temperature superconductivity cannot now be found before 1990.

John Maddox

Cell biology

Reception and transmission

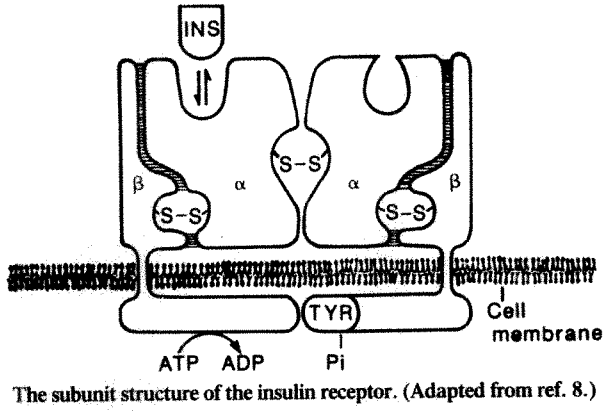
Asser S. Andersen

THE activity of cell-surface receptors is in many cases controlled by their association state in the membrane, and the association state is in turn regulated by the attachment of the ligand. Rutter and his colleagues in San Francisco now show[1] that this ligand-dependent association can be mimicked in free solution by the extracellular domain of the insulin receptor. The precise relationship between receptor association and the biochemical events that the receptor controls in the cell is less well-defined, but here too there are some new pointers.

Cell biology and endocrinology have both come a long way since the first evidence that insulin can regulate the activities of a cell without actually going inside was met with incredulity. Signal transduction has now become almost a discipline of its own, and the insulin–insulin receptor system remains the prime example. The way it works is as follows. The insulin receptor, embedded in the cell membrane, is a heterotetramer of four subunits ($\beta\alpha\alpha\beta$) held together by disulphide bonds (see figure). Insulin binds tightly to the extracellular α-subunits, causing the activation of a tyrosine kinase activity which resides in the intracellular domain of the β-chains. The β-subunits then phosphorylate each other, leading to a rise in kinase activity. A still largely uncharacterized regulatory cascade is set in train, involving the phosphorylation of several cytoplasmic proteins. A review of these processes published some years ago was entitled "Insulin binds, and then something happens": although the frontiers of ignorance have been pushed back since then, there are areas that are still shrouded in fog.

To cell biologists, the question of greatest interest must still surely be how the binding of the insulin molecule activates the receptor, and here there has recently been great progress, much of it based on the introduction of alien insulin receptors into mammalian cells in culture. The α- and β-chains are synthesized *in vivo* on a single messenger RNA and then proteolytically separated and inserted into the cell membrane.

Johnson *et al.*[1] have transfected cells of a mammalian (CHO) cell line with complementary DNA encoding either the whole $\alpha\beta$ ensemble or truncated versions lacking most of the β-chain, so as to eliminate both the transmembrane and kinase domains. These secreted 'ectodomains' emerge with the correct quaternary structure (two disulphide-linked α-chains, each with its vestigial β-chain), and properly finished in terms of glycosylation. They bind insulin with fully developed affinity (which is not surprising, for the insulin-binding domain is intact). The striking observation of Johnson *et al.*[1] is that when insulin is added to a solution of purified ectodomains, these entities associate into small clusters or, depending on the concentration, long aggregates, which branch or loop back on themselves. The authors infer from the similar effect of epidermal growth factor (EGF) that the aggregation is a consequence of mere occupancy of a ligand site, rather than a specific effect of insulin. (On the other hand, although the EGF and insulin receptors are structurally related, and EGF does perturb biochemical responses to insulin in some cell types[2], it has never been found actually to compete with insulin on the insulin receptor.)



The subunit structure of the insulin receptor. (Adapted from ref. 8.)

Other groups have also tinkered with insulin receptors. In particular, Maegawa *et al.*[3] have introduced a truncated recombinant version of the human insulin receptor, devoid of kinase activity, into rat fibroblasts which have the normal (or even an increased) complement of functional receptors. It turns out that these alien and silent receptors do not interfere at all with the action of the endogenous receptors, up to the point of phosphorylation of the β-chain and stimulation of its kinase activity. But thereafter, things go wrong: the endogenous receptor fails to phosphorylate its cytoplasmic substrates, and the transfected cells show none of the normal physiological responses to insulin binding. An interesting (but by no means the only) explanation could be that receptor aggregates are needed to generate biological activity, and complexes of the active with the inactive sort are not functional. The CHO cells that make human insulin receptors in addition to their own[1] have yielded another morsel of evidence for the importance of receptor aggregation[4]: when there are more receptors, fewer insulin molecules are needed to generate a given response. This implies that the closer the receptors are in the plasma membrane the better they amplify the binding signal.

Using the more homespun methods of traditional biochemistry, the groups of Pessin[5] and of Kahn[6] have cast new light on the early steps of insulin action. Sweet *et al.*[5] find that mild reduction causes the heterotetramer to dissociate into $\alpha\beta$-dimers. When incubated with insulin under oxidizing conditions, these revert to the tetramer, showing again that extracellular ligand regulates association state.

Shoelson *et al.*[6] re-examine an old mystery — that trypsin can mimic the effects of insulin in various cells. Their observations suggest that a proteolytically induced relaxation of the α-subunit releases the steric constraints which normally keep the β-subunits from phosphorylating each other. Such a model is consistent with the properties of another engineered fragment: a truncated insulin receptor, lacking the insulin-binding domain, confers the complete gamut of insulin responses on the cell in which it is expressed, without the need for insulin[7]. So it seems that the function of the α-subunit is to keep the β-subunits dormant when there is no insulin to press the switch.

All this is progress indeed, but questions remain to be answered. For instance, when receptors are isolated from most cell types they appear to be exclusively in the $\alpha_2\beta_2$ form and phosphorylation, which is presumed to take place within the tetramer on binding of insulin[5], requires no further aggregation. What then are we to make of the aggregates of Johnson *et al.*? Is it possible that EGF can modulate the cell's response to insulin by altering the association of the insulin receptors? Does phosphorylation or some other kind of interplay between heterotetramers control a later event in the insulin response? □

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Computation

Highly distributed processing

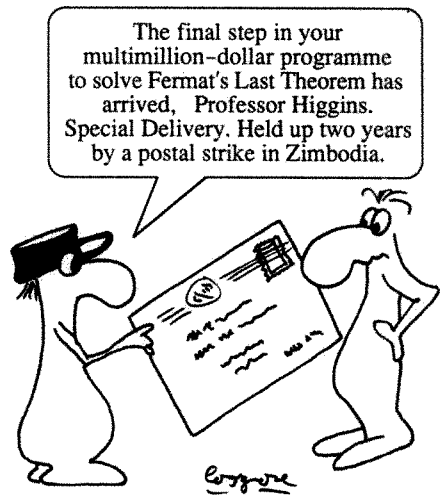
Ian Stewart

WHAT is the best way to obtain raw computational power? A massive central supercomputer or a network of smaller machines? Both have their passionate devotees. A recent calculation in number theory has given a new meaning to the phrase 'distributed processing': it illustrates the possible advantages of a network for problems that can be broken into independent pieces. A 100-digit number, carefully screened for maximum difficulty, has been resolved into prime factors by the communal efforts of a dozen mathematicians in three countries using 400 separate computers simultaneously and communicating by electronic mail. The marathon calculation widely reported in the popular press (see *Nature* **335**, 658; 1988) was organized by Mark S. Manasse of the Digital Equipment Corporation's research centre at Palo Alto and Arjen K. Lenstra of the University of Chicago. It took less than a month: a supercomputer working non-stop would have taken at least a week and cost a great deal more.

The factorization of large numbers into primes used to be of purely theoretical interest. Today, however, the problem has important applications in cryptography and cryptanalysis. A well known method of encoding sensitive information, the RSA (Rivest–Shamir–Adelman) system, relies for its security on the conjectured difficulty of resolving an arbitrary number into its constituent primes. Any improvement in factorization methods is thus automatically of interest in a much wider sphere. The communal effort under discussion is in a sense most noteworthy for its *lack* of novelty. The actual method employed, known as the quadratic sieve, was invented by Carl Pomerance in 1982 (*Computational Methods in Number Theory Part I* (eds Lenstra, H. W. Jr & Tijdeman, R.) Mathematisch Centrum Tract **154**, Amsterdam; 1982), and was rapidly accepted as one of the fastest methods available.

The first thing to understand about prime factorization is that the most obvious method, trial division of the number N by each possible prime in turn up to the square root, is hopelessly inefficient for numbers with more than just a few digits. The number of primes less than $\sqrt{N}$ is approximately $2\sqrt{N}/\log N$, so the number of trials needed for a 100-digit number is roughly $2 \times 10^{50}/(50\log 10) = 1.7 \times 10^{48}$. At a million trials per second the computation would take about 5×10^{34} years, or at least 10^{27} times the age of the Universe. Variations on this technique, such as strategies for searching specific ranges of primes, like starting at $\sqrt{N}$ and working backwards, suffer from the same defect.

The solution is to apply more subtle methods, based on results in number theory. Suppose we wish to find the prime factors of a number N. If we can find just one of them, we can divide it out and repeat the process on the much smaller number that results. So the real problem is to find *some* prime factor. One approach is to look for two perfect squares whose



difference is a multiple of N. Let x^2 and y^2 be these squares. Because $x^2-y^2 = (x-y)(x+y)$, any prime factor of N must divide either $x-y$ or $x+y$. Suppose for the sake of argument that it divides $x+y$; then it also divides the highest common factor of $x+y$ and N. There is a very efficient method, known as the euclidean algorithm, for computing this highest common factor, which in general is much smaller than N. Because it is smaller, we can find its prime factors relatively easily; but these are also prime factors of N. The strategy is thus very much one of 'divide and conquer'.

It's a neat trick — but how do we find x and y? Michael Morrison and John Brillhart (*Mathematics of Computation* **29**, 183–205; 1975) realized that suitable candidates for x and y could be found by using the continued fraction for $\sqrt{N}$, a sequence of fractions p/q that approximate $\sqrt{N}$ ever more closely. Their proposal was to run along this sequence of fractions waiting for the denominator q to become a perfect square. When this happens, suitable x and y can easily be found. But there is a much better way. Instead of waiting for a square denominator to turn up, we can try to create one. Some upper limit is chosen and each denominator is checked to see whether all of its prime factors lie below this limit. If so, its factorization is stored as a sequence of zeros and ones: 0 if the corresponding prime factor occurs t even power, 1 if it occurs to an odd po In this way a huge matrix is built up, these sequences as its rows.

Some subset of all these denomina multiplied together, will form a squa provided that the corresponding s ences have, between them, an even n ber of ones in each column. This ca checked quickly by standard techni of linear algebra, known as gaus elimination. Having found a combina of denominators whose product is a fect square, numbers x and y such N divides x^2-y^2 are again easy to find. is the basic idea: the full-blooded quad sieve incorporates several further re ments, including strategies to cut shor computation if it is getting nowhere. detailed description see *Prime Num and Computer Methods for Factoriza* by Hans Riesel; Birkhäuser, Boston, 19

Manasse and Lenstra's collabora were from the United States, the Net lands and Australia. The matrix requ to apply the quadratic sieve turned ou have 50,000 rows and columns, a tot 250 million entries. The computatio the matrix was split into 400 pieces, results were assembled by electronic at Palo Alto, and the final gaussian el nation led to the discovery that the n ber had two prime factors, with 40 an digits. There are so many computers that much of their time is effectively f especially for low-priority jobs runnin background', and the tale bears dram witness to the capabilities of a networ small machines running in parallel.

The quadratic sieve is a highly refi and extremely clever technique, but by now become fairly standard. So sense the method is an example of br rather than brains. The particular styl brawn, large-scale international coop tion, is seldom applied in such an or nized manner. On the other hand, whole of mathematics is a large-s international cooperation, whose custo ary methods of communication are ofte good deal slower than electronic mail.

By pushing the present approach to limits, factorizations of 110- or perh 120-digit numbers might be achieved. factorize an arbitrary 150-digit numb say, still looks out of the question, the only hope is a completely new id This is not inconceivable; for exam Hendrik Lenstra (of Amsterdam Univ sity) recently suggested a new appro involving so-called elliptic curves (see News and Views article in *Nature* **325**, 1 1987), which can sometimes be extrem efficient. But no known method wo efficiently for the awkward cases as well the nice ones. In the world of prime f tors, nobody yet knows whether brain ultimately prove superior to brawn.

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RNA processing

Splicing a spliceosomal RNA

David A. Brow and Christine Guthrie

Two fundamental questions that captivate the interest of those who study RNA processing are how interrupted genes acquired their introns and how the removal of introns from RNA is catalysed. Tani and Ohshima report on page 87 of this issue[1] a remarkable finding that may bear on both questions: they identify an intron within a gene encoding a spliceosomal RNA. The spliceosome is the structure responsible for removal of introns from nuclear pre-messenger RNA (Fig. 1). It is assembled from the pre-mRNA, four small nuclear ribonucleoprotein complexes (snRNPs) called U1, U2, U5 and U4/6, containing five small nuclear RNAs (snRNAs), and an as yet unknown number of additional proteins (see ref. 2 and the News and Views article[3] by M. R. Green). Because the intron discovered by Tani and Ohshima has the distinctive structure of nuclear pre-mRNA introns, it seems in this case that an snRNA is spliced by the very apparatus of which it is an important component.

The intron interrupts the single-copy gene encoding U6 snRNA in the fission yeast *Schizosaccharomyces pombe*, and contains perfect matches to the consensus sequences that have been identified at the splice sites and at the site of branch formation in nuclear pre-mRNA. Tani and Ohshima point out[1] that this strict adherence to the consensus motif, in an organism where these sequences are often rather divergent, suggests the U6 intron is under selective pressure for efficient removal. Indeed, Tani and Ohshima observe only fully spliced U6 snRNA *in vivo* under normal growth conditions. Although the participation of U6 in the removal of the U6 intron can as yet only be inferred, a consideration of the possible origin of this intron has interesting implications for the general role of U6 snRNA in splicing.

It is the snRNAs, rather than the proteins, that are believed by many to be the main catalytic elements of the spliceosome. This notion follows from the discovery of another class of RNAs, the group II self-splicing pre-mRNAs, in which excision of the intron and ligation of the remaining exons occurs via a pathway that is mechanistically very similar to that of nuclear pre-mRNAs, but does not require proteins[4,5]. Indeed, it has been suggested that the spliceosome evolved from such autocatalytic RNAs[6]. Thus, in the study of spliceosome-mediated intron removal, much effort is being directed towards attributing specific catalytic functions to the snRNAs.

U6 snRNA is by far the most highly conserved spliceosomal RNA[7], and so is expected to have a fundamental role in splicing. Yet, unlike the U1, U2 and (probably) U5 snRNA-containing snRNPs, which recognize the conserved intron elements (Fig. 1), the function of the snRNP containing U4 and U6 snRNAs is unknown. U4 and U6 are strongly associated with one another by base-pair interactions that form two contiguous stems (Fig. 2, stems I and II). Curiously, these stems lie within the most highly conserved segment of U6, consisting of 49 nucleotides with at least 80 per cent identity between yeasts and mammals. Normally, paired bases exhibit phylogenetic covariation, changing their identity but maintaining complementarity (for example, A–U changes to G–C). This paradox could be resolved by the surprising observation that the stable U4/6 interaction is disrupted during splicing *in vitro*, as judged by the loss of U4 from the spliceosome before or during the first nucleolytic step[8-10] (Fig. 1). Thus, the conservation of U6 within the stems could reflect an additional role for this region when it is unpaired. Indeed, the timing of U4 dissociation with respect to the generation of splicing products suggests that this highly conserved region of U6 may be involved in catalysis. U4 could thus be viewed as an anti-sense RNA that negatively regulates U6 activity.

The intron identified by Tani and Ohshima is located precisely in the middle of the highly conserved region of U6, bifurcating interaction stem I (Fig. 2). Its presence in a U6 gene is particularly surprising as this is the first example of a pre-mRNA-like intron in an RNA polymerase III transcript[11,12]. The fact that half a dozen

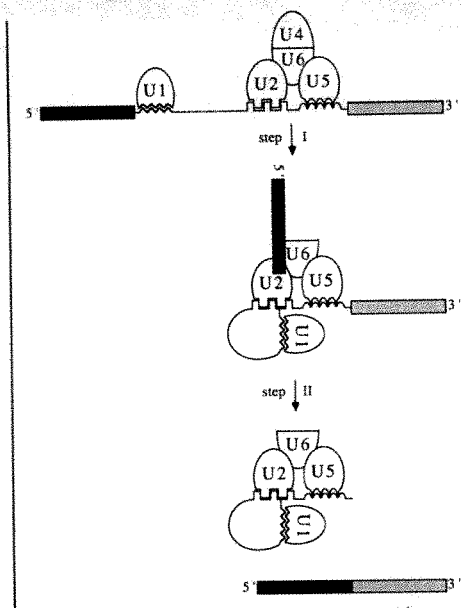


Fig. 1 Nuclear pre-mRNA splicing pathway. The 5′ and 3′ exons are represented as dark and light boxes, respectively, and the intron as a thin line. Top, fully assembled spliceosome, with U1, U2 and U5 snRNA-containing snRNPs interacting with conserved sequences at the 5′-splice site, branch point and 3′-splice site, respectively. Middle, during or before the first nucleolytic step of splicing, U4 dissociates from the spliceosome, which now contains free 5′ exon and the branched 'lariat' intermediate. Bottom, step II produces spliced mRNA and free intron lariat, still bound by snRNPs. (Contacts between snRNPs do not imply proven associations.)

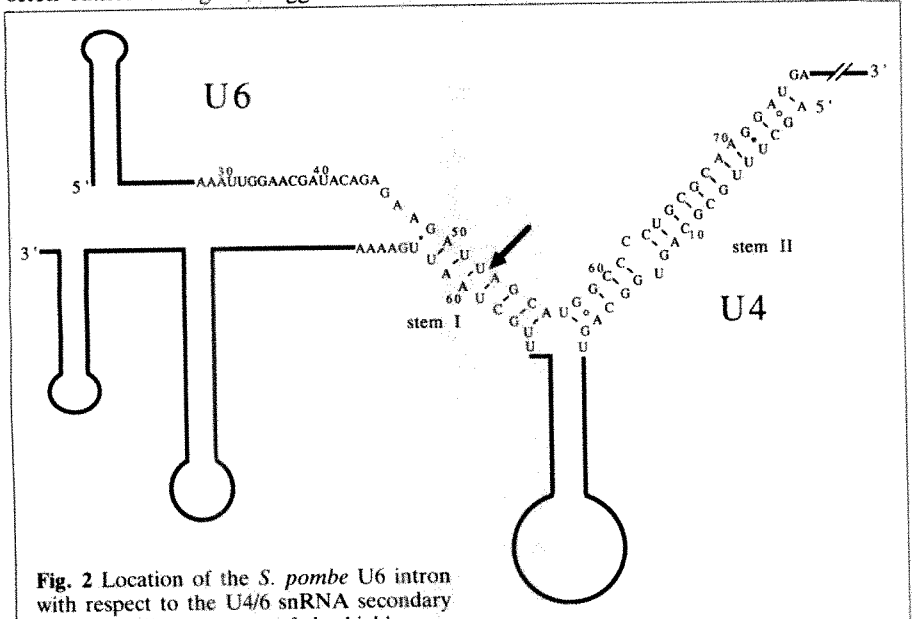


Fig. 2 Location of the *S. pombe* U6 intron with respect to the U4/6 snRNA secondary structure. The sequence of the highly conserved region of U6 (from mammals), and the region of U4 with which it pairs[7], are shown. Other stems are shown as adjacent parallel lines. Arrow, site equivalent to the *S. pombe* intron insertion site described by Tani and Ohshima[1].

other sequenced U6 genes (including two from the budding yeasts) contain no intron, together with the obvious 'chicken-or-egg' problem presented by an intron in a spliceosomal RNA, suggest that the intron was not present in the progenitor U6 gene, but may have been acquired more recently in evolution. Given that the U6 intron does not appear to be a transposable element[1], how did it arise?

An intriguing possibility is that the *S. pombe* U6 intron arose from a mishap during pre-mRNA splicing. Perhaps, during its excision from pre-mRNA, an intron inserted into the U6 RNA molecule present in the spliceosome. The resulting product was presumably reverse-transcribed and its complementary DNA then replaced the chromosomal U6 gene. If this scheme is correct, the site of intron insertion would probably reflect the proximity of that part of U6 to the catalytic centre of the spliceosome. The picture of U6 that emerges is of a central, possibly catalytic site flanked by conserved sequences positioned to interact, directly or through other snRNPs, with the pre-mRNA near the 5′ and 3′ splice sites.

Insertion of an excised intron into RNA is not unprecedented: it has recently been found that autocatalysed splicing of a group I pre-ribosomal RNA (which proceeds by a mechanism somewhat different from that used by the group II and nuclear pre-mRNA introns[6]) can run backwards, with the intron reinserting at the site of its excision (S. Woodson and T. Cech, personal communication). Although the origin of the intron discovered by Tani and Ohshima is a matter for conjecture, a significant step in advancing these ideas from the realm of speculation may come from further sequence analysis. Identification of an intron at the same site in the U6 gene of an organism unrelated to *S. pombe* would support the proposed mechanism of insertion and its implications for U6 function. □

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Submillimetre astronomy

Different views of the Universe

Glenn J. White

ONE of the few remaining unexplored regions of the electromagnetic spectrum accessible to ground-based astronomical observations lies at wavelengths between 1 and 0.3 mm. Using the new generation of large submillimetre-wave telescopes which have recently been completed in Hawaii, Europe and Chile, astronomers have been actively studying a wide range of astrophysical problems related to the Solar System, the physics of star formation, the chemical and dynamical evolution of the interstellar medium, the structure and evolution of galaxies, and the nature and energetics of active galactic nuclei and quasars. This spectral region is well suited to the study of gas and dust in the interstellar medium, particularly in its ability to sample regions which are highly opaque at optical wavelengths. The first results from these new telescopes, especially those relating to the interstellar medium, were discussed at two recent meetings*.

An accurate determination of the mass of material in the interstellar medium is crucial to many astrophysical studies, including those of the structure and dynamics of molecular clouds and their subsequent formation of stars. Most of this mass is in the form of hydrogen, which remains very difficult to observe from the surface of the Earth. Consequently it is necessary to infer its presence from observations of other molecules such as carbon monoxide, which undergo frequent collisions with it. These collisions excite rotational (J) transitions in the molecular species, which are observable throughout the millimetre and submillimetre wavelength part of the spectrum. As part of the continuing controversy about the $N(H_2)/W(CO)$ ratio (column density of H_2 over integrated intensity in the CO $J=1$–0 transition) in interstellar gas in our Galaxy and external galaxies, new observations indicate that the value of this ratio is about six times larger in the Large Magellanic Cloud than in our own Galaxy (T. Dame, Harvard–Smithsonian Center for Astrophysics), and up to 25 times larger in the Small Magellanic Cloud (M. Rubio, Harvard–Smithsonian Center). To explain these discrepancies, it was suggested that scattering of CO radiation in the low-density envelopes of clouds may in part account for the discrepancies between $N(H_2)$ determinations from CO, ^{13}CO and $C^{18}O$ in galactic clouds (M. Guélin, Institut Radio Astronomie Millimétrique).

In addition to the hydrogen gas, about 70 other molecular species have been detected in the interstellar medium as minor constituents. These are subject to a complex range of chemical interactions, which also influence the thermal balance of the gas and ultimately the structure and stability of molecular clouds. With the extremely high angular and spectral resolution now available in the millimetre and submillimetre wavelength region, detailed studies are possible which trace variations of both chemistry and excitation throughout molecular clouds. New evidence shows that ultraviolet radiation can penetrate interstellar clouds to a much greater extent than previously realized, because of the clumpy nature of material in molecular clouds. This can lead to an increase in the rate of photodissociation of the molecules, and modification of the local chemical equilibrium. These results, based on submillimetre wavelength observations of C I and C II (neutral and singly ionized carbon) emphasize the increasing importance of high-frequency observations (J. Stutzki, Max Planck Institut für Astrophysik, Garching).

One important consequence of this higher radiation field is to speed up the chemical processing and evolution of molecular clouds (W. Langer, Princeton University). The models, however, are still unable to explain all the recent observational estimates of the abundances of various molecules. Consequently, the importance of time-dependent gas-phase chemistry (E. Herbst, University of Cologne), and grain-surface reactions, particularly for hydrogenated amorphous carbon (W. Duley, York University) were discussed to account for the long-chain hydrocarbon molecules observed in regions like the Taurus molecular cloud.

After the gravitational collapse of a molecular cloud, stars may ultimately be born out of the gas, with some of the residual debris going on to form 'planetary' systems. Although it is perhaps remarkable that ground-based observations of rather 'average' stars in the Galaxy can still hold many new surprises, recent observations have confirmed the presence of dust shells around stars such as Vega, α PsA and β Pic (E. Becklin, University of Hawaii). The submillimetre wavelength fluxes, however, fall well below those predicted from an extrapolation of the Infrared Astronomical Satellite (IRAS) measurements, suggesting that the particles are mostly less than 100 μm diameter. The most likely explanation is that they have been generated since the formation of the star, probably as a consequence of cometary or asteroid collisions.

For molecular clouds to collapse to form stars, a delicate balance between

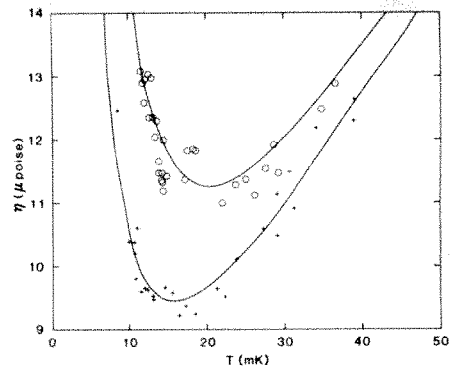
* *The Physics and Chemistry of Interstellar Molecular Clouds*, Zermatt, 22–25 September 1988 and *Millimetre and Submillimetre Astronomy*, Kona, Hawaii, 3–6 October 1988.

their internal pressure and the opposing force of gravity regulates the rate at which stars will be formed. Until recently it has been extremely difficult to estimate the strength of magnetic fields inside dense regions, although this has now become possible at millimetre and submillimetre wavelengths, in particular by measuring the amount of polarized radiation emitted from the interstellar dust. Measurements of the magnetic fields in several molecular clouds suggest that the magnetic contribution to the internal motions and energy of many of these clouds is crucial for cloud dynamics, cloud evolution and star formation (P. Myers, Harvard–Smithsonian Center; R. Hildebrand, University of Chicago). In addition it has been suggested that hydromagnetic waves may be responsible for inducing density fluctuations throughout the interstellar medium (R. Pudritz, McMaster University), and examples of large-scale filamentary and ordered structures can now be seen in extensive maps of regions such as the Orion molecular complex (J. Bally, AT&T Bell Laboratories). The size scales of these large structures, which can extend over many tens of square degrees on the sky, emphasize the unique role which can be played by small telescopes, whose large beams make it possible to map extensive areas of the sky rapidly (P. Thaddeus, Harvard–Smithsonian Center).

The Galaxy is a large place, and it remains difficult to build up a detailed view of the large-scale processes occurring during stellar formation. However, the physics of star-formation across a whole galaxy can be uniquely viewed in nearby face-on spiral galaxies. Using a millimetre wavelength interferometer, new observations of M51 have been made which reveal the presence of giant molecular complexes having sizes of around 400 parsecs and masses greater than 10^7 solar masses. The velocity structure of the molecular material shows systematic gradients across individual galactic spiral arms, where the star formation efficiency is also found to be strongly enhanced. This suggests that density waves are responsible for triggering star formation on a galactic-wide scale (S. N. Vogel, Rensselaer Polytechnic Institute: see Vogel, S.N., Kulkarni, S.R. & Scoville, N.Z. *Nature* **334**, 402–406; 1988).

The millimetre and submillimetre wavelength regions are important to the study of objects such as ultraluminous galaxies. These objects, first detected by IRAS, may in some cases go on to form quasars. Using a synthesis of recent results and new observations in the CO line, it was suggested that the collision, or merger, of two spiral galaxies, can result in removal of angular momentum and allow an infall of gas towards their centres. This rapid accretion leads to a star burst in the nucleus, and then the aggregation of gas and stars into a massive central stellar cluster. The subsequent nucleation of this core, and a continued infall of material from the outer parts of the merger may lead on to the formation of an active galactic nucleus or quasar (N. Z. Scoville, California Institute of Technology). □

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Measurements of the viscosity η of a 0.1-per-cent ^{3}He–^{4}He solution as a function of temperature T in zero magnetic field (+) and in a magnetic field of 10 tesla (○). The lower solid curve represents a fit of theory to the data with the strength of the interaction between the ^{3}He atoms as an adjustable parameter; the upper curve is a prediction by the same theory without any further adjustable parameters. (From Owers-Bradley *et al.*.)

Low-temperature physics

Viscosity of ^{3}He–^{4}He solutions

P.V.E. McClintock

Liquid helium thickens slightly when subjected to a strong magnetic field at very low temperatures. The latest measurements of this intriguing effect, which occurs only in liquid ^{3}He–^{4}He isotopic mixtures or in pure liquid ^{3}He, are reported in a recent issue of *Physical Review Letters* (**61**, 1619–1622; 1988) by J.R. Owers-Bradley and colleagues at Nottingham University.

The new experiments were performed on a dilute solution containing 0.1 per cent of the rare helium isotope ^{3}He dissolved in the common isotope ^{4}He. The properties of liquid ^{3}He and liquid ^{4}He at low temperatures are markedly different, and a solution of this kind is a rather strange material. Liquid ^{4}He, a so-called boson system in which there is no restriction on the number of particles that may occupy any given energy state, becomes superfluid below a temperature of 2.17 K: it loses all its viscosity and objects can move through it without experiencing the drag found in conventional liquids.

Liquid ^{3}He, on the other hand, is a fermion system in which there is a rigorous restriction that forbids more than one particle from simultaneously occupying any given energy state. (It too becomes superfluid, but must be a thousand times colder before the transition occurs.) When dissolved in ^{4}He, ^{3}He atoms move effortlessly through the superfluid. In fact, the superfluid ^{4}He just constitutes a kind of ethereal background, often termed a mechanical vacuum. The ^{3}He atoms in it form an almost ideal gas and, for most purposes, the presence of the superfluid background can be ignored.

Because of the restriction on the occupation of energy states, it is characteristic of fermion systems that they possess a great deal of energy, even at 0 K. The system tries to get into the lowest allowed energy configuration, which means that every permitted state is occupied from zero up to a maximum energy known as the Fermi energy, E_F; all states above E_F are empty. The magnitude of E_F depends on the density of fermions and, in the case of a ^{3}He–^{4}He solution, can conveniently be adjusted simply by changing the concentration of ^{3}He.

The ^{3}He nuclei carry tiny magnetic moments which, according to quantum mechanics, align either with or against an applied magnetic field, the two alignments having different energies. When a magnetic field is first applied to the cold liquid, half of the ^{3}He atoms suddenly have their energy increased relative to the other half so that, in effect, each group momentarily has a different Fermi energy. Relaxation processes then occur in which some of the higher-energy atoms make transitions to the lower-energy aligned states, until the value of E_F is again common to both groups. The liquid becomes polarized, there being more atoms aligned with the field than against it. At sufficiently low temperature, the degree of polarization achievable by this 'brute force' method depends on the relative magnitudes of the magnetic and Fermi energies. By using a dilute ^{3}He–^{4}He solution, making E_F relatively small, Owers-Bradley *et al.* obtain a much higher degree of polarization in a given magnetic field than was the case in the earlier experiments of, for example, G.A. Vermeulen *et al.* (*Phys. Rev. Lett.* **60**, 2315–2318; 1988) using pure liquid ^{3}He.

Because the interactions between pairs of ^{3}He atoms depend on whether their nuclear magnetic moments are parallel or antiparallel to each other, the properties of a polarized ^{3}He–^{4}He solution are expected to differ markedly from those of an ordinary unpolarized one. The interaction is stronger between pairs of atoms whose moments are antiparallel, and these

obviously become fewer as the solution polarizes. The mean free path of the atoms consequently increases with polarization, generating corresponding changes in the transport coefficients, including an increase in viscosity. From the measured magnitude of the change in viscosity it should be possible to derive parameters of the interaction between the ^{3}He atoms.

Following a technique described by D.S. Greywall and M.A. Paalanen (*Phys. Rev. Lett.* **46**, 1292–1295; 1981), Owers-Bradley *et al.* used measurements of second sound — a density wave in the gas of ^{3}He atoms — to deduce the viscosity. They investigated standing waves of second sound in a resonant cavity and, after making appropriate allowance for other dissipative processes, determined the viscosity of the liquid from the measured widths of the resonances. The results (see figure) show clearly that the viscosity increases on application of the field, the increase becoming substantially larger as the temperature is reduced. The results should be more accurate and reliable than the earlier ones of Greywall and Paalanen who worked with a resonator which produced a distorted line shape owing to a hole in the cavity wall needed to assist cooling of the sample inside. In the new resonator, the inner wall is made of sintered silver, whose large surface area allows the sample to be cooled directly without any need for a hole to the exterior refrigeration arrangements.

An interesting feature of the results is that, unlike in an earlier experiment by D.A. Ritchie *et al.* (*Phys. Rev. Lett.* **59**, 465–468; 1987), there seems to be no evidence of 'slip' at the walls: rather, there was a boundary layer of effectively stationary ^{3}He at the walls, as would be expected with an ordinary viscous fluid. The authors suggest the difference may arise because Ritchie *et al.* used a smooth-walled chamber, quite different at the microscopic level from the rough, porous, sintered silver walls of the present experiment.

The lower curve of the figure represents a fit of a theory, based on ideas originally developed by E.P. Bashkin and A.E. Meyerovich (*Adv. Phys.* **30**, 1–92; 1981), to the zero-field viscosity measurements, using the strength of the interaction between the ^{3}He atoms as an adjustable parameter. The agreement is excellent and in itself suggests that the deduced form of the interaction is probably correct. The real test, however, is seen in the upper curve which is a prediction of the behaviour at 10 tesla without recourse to any additional adjustable parameters. Again, the agreement is excellent, apparently confirming the validity both of the theory and of the interaction deduced by fitting it to the zero-field data. □

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Sickle cell anaemia

A simple disease with no cure

L. Luzzatto and P. Goodfellow

MOLECULAR biology has explained the basic defect in sickle cell anaemia and has provided the tools for prenatal diagnosis, but the treatment of afflicted patients undergoing crisis has not correspondingly improved. For the pessimist, this fact poses the general question of the efficacy of molecular approaches for treatment of genetic disease; and this echoes previous debates about the reductionist approach of molecular biology. Be that as it may, a positive step was taken by the Wellcome Trust in the sponsoring of a Sickle Cell Workshop, held in London on 4 November 1988, to consider new approaches to the treatment of sickle cell anaemia.

No genetic disease could be simpler than sickle cell anaemia. A single base pair in the β-globin gene is mutated from adenine to thymine producing a corresponding change in the sixth amino acid of the protein from glutamic acid to valine. Under conditions of low oxygen tension, the abnormal deoxyhaemoglobin polymerizes and eventually leads to a change in red blood cell shape termed sickling. When distorted red blood cells occlude small blood vessels, this can result in 'crisis', a term that dramatically describes a wide spectrum of events, from relatively brief painful episodes to massive thrombosis leading to infarction in bone, lung or brain. The organ damage is potentially crippling and the crisis may be life-threatening.

Following the theme of "know your enemy", G. Serjeant (MRC Laboratory, Jamaica) described the natural history of sickle cell anaemia. The trigger for crisis is not clear, but its onset is often associated with a drop in environmental temperature (Redwood, A.M. *et al. Br. med. J.* **i**, 66; 1976). Exposure to cold may divert blood flow from the bone marrow, causing local necrosis and 'bone' pain. Exploration of this hypothesis will require quantitation of the environmental factors triggering crisis and measurements of blood flow to the bones of patients suffering crisis. The entire physiology of the microcirculation in bone and bone marrow requires investigation, especially because the rheology of blood is affected by the presence of sickled cells and also by the numbers of white cells (I. Smaje, Wellcome Trust), which are often increased during crisis.

Although all patients with sickle cell anaemia have exactly the same genetic defect, there is wide variation in clinical expression of the disease. D. Weatherall (Oxford University) considered genes that modify the phenotype. Some of this variation results from the coexistence of a deletion in another globin gene, α-thalassaemia (see Dover, G.J. *et al. Blood* **69**, 341; 1987). Thus a typical mendelian disorder has some of the features of a polygenic disorder. Other hypothetical compensatory mutations may affect the intraerythrocytic level of diphosphoglycerate (DPG), the main physiological regulator of the oxygen–haemoglobin dissociation curve. In other chronic anaemias, an increase in DPG levels improves oxygen delivery to tissues (Torrance, J. *et al. New Engl. J. Med.* **283**, 165; 1970); but in sickle cell anaemia this adaptive response backfires because the extra deoxygenation of haemoglobin favours sickling. Another component favouring sickling could be a *p*H-dependent ouabain-insensitive potassium channel that is apparently more active in the young red cells of sickle cell anaemia patients (C. Ellory, Oxford University). This channel may contribute to dehydration of red blood cells and the consequent promotion of irreversibly sickled cells. Both this potassium channel and the enzyme diphosphoglycerate mutase, which regulates the DPG level, are potential targets for drug therapy.

Treatment strategies can be broadly divided into five categories: anti-sickling agents; vasoactive drugs; promotion of persistence of fetal haemoglobin; bone marrow transplantation; and gene therapy. The first three approaches have failed so far, but there is increasing interest in the last two. Transplantation is available only to patients who have a suitable sib donor and the risks associated with the procedure and with graft-versus-host disease are still too high for this approach to become generally applicable in the treatment of sickle cell anaemia. But it is worth noting that bone marrow transplantation has been used successfully to treat about 200 children suffering from the more severe anaemia, thalassaemia major.

Gene therapy would avoid the problems associated with transplant rejection and graft-versus-host disease. Two approaches can be envisaged: replacing the mutated β-globin gene by homologous recombination; or insertion into the genome of haemopoietic cells of an additional copy of the normal gene. The ideal approach is the former, but it requires a substantial improvement in the isolation and culture of pluripotent stem cells as well as in the efficiency of homologous recombination. The second approach also requires technical advances in stem cell culture and the development of suitable

gene-transfer methods. In the meantime, the recent identification of a control element 60 kilobases upstream of the β-globin gene (F. Grosveld, National Institute for Medical Research, London) has solved the problem of obtaining high-level, tissue-specific expression of human β-globin in transgenic mice. Nevertheless, if gene therapy is going to be a cure for sickle cell anaemia (and many other diseases), a serious investment of resources must be made in this area.

Sickle cell anaemia has always been the paradigm for molecular analysis of genetic disease and the disappointing progress in treating it could be a warning for those studying other diseases. As is clear from the example of sickle cell anaemia, avoidance of this fate will require continued studies on the natural history of diseases and the physiological interactions in the whole organism: perhaps a true anti-sickling agent will then turn up.

The development of novel treatment strategies could be greatly facilitated by animal models, and it would seem to be a high priority to adopt the new techniques of embryonic stem cell manipulation to create a mouse model of sickle cell anaemia (see Rubin, E.M. *et al. Am. J. hum. Genet.* **42**, 585; 1988). In some cases, mutations in the mouse models have failed to mimic directly the human disease. Notable examples include deficiency of the enzyme HPRT for Lesch–Nyhan disease and the *mdx* mouse models for Duchenne muscular dystrophy, respectively. But this serves only to emphasize the importance of physiological studies and provides an opportunity for discovering key steps in the disease process. Finally, if somatic gene therapy is going to be a viable approach for treatment of genetic disease, much more must be learned about the biology of stem cells, be it in muscle, brain or bone marrow. □

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Geological boundaries

Extinctions and carbon cycling

M.L. Delaney

ARE mass-extinction events causes or consequences of changes in global carbon cycling? Reports on marine sequences by Holser *et al*[1], Gruszczyński *et al.*[2] and Zachos *et al.*[3] on pages 39, 64 and 61, respectively, of this issue conclude that substantial shifts in carbon partitioning among and within major sedimentary reservoirs occurred before, during and after extinction events defining the boundaries of the Mesozoic era. But the nature of carbon cycling associated with these mass extinctions, as recorded in the oceanic carbon istope composition, is unique to each boundary. The Permian/Triassic mass extinction, about 250 million years ago (Myr), followed a prolonged interval of greatly increased net organic carbon oxidation. By contrast, the Cretaceous/Tertiary extinction (about 66 Myr) apparently resulted in the rapid cessation of oceanic primary productivity. After both boundaries, despite their different natures, recoveries of oceanic carbon isotope systems to more typical conditions took a substantial time.

Organic matter, formed in oceanic surface waters where light and nutrients are available for primary productivity, has a lower ratio (by about 2 per cent) of the rare, heavier stable carbon isotope (^{13}C) to the more abundant one (^{12}C) than the dissolved inorganic carbon. Therefore, dissolved inorganic carbon in surface waters, where organisms have used most of the available dissolved nutrients and preferentially taken up the lighter carbon istope, is relatively enriched in ^{13}C. The ^{13}C-depleted organic matter falls through the water column and is recycled back to its dissolved, inorganic constituents at depth, giving the dissolved inorganic carbon in deep waters a relatively low ratio of ^{13}C to ^{12}C. This surface-to-deep difference in the ratio of the two forms of carbon is thus generated by the cycle of primary production in surface waters and organic matter regeneration in deep waters. Consider a case where primary productivity in surface waters was greatly reduced (while nothing else about the ocean changed); $^{13}C/^{12}C$ ratios in surface and deep waters would become more similar, with the surface-to-deep gradient in carbon isotope ratio reduced.

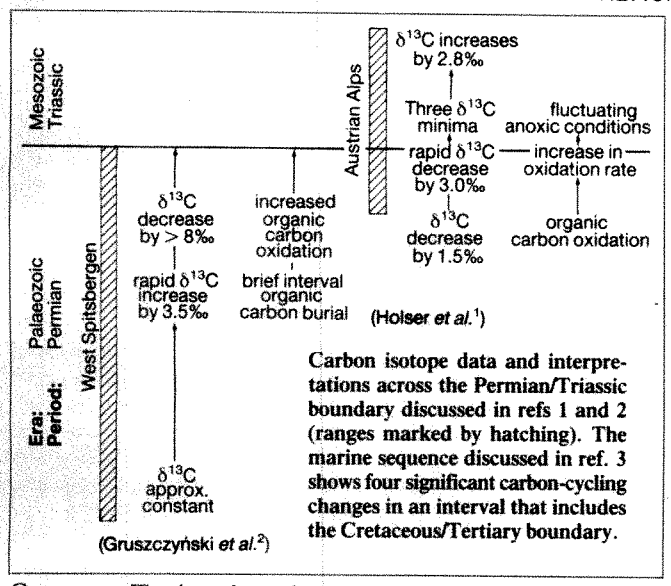
Over longer timescales, the important carbon sources and sinks for the ocean as a whole are weathering of material from and burial of material into two sedimentary reservoirs: organic carbon, relatively depleted in the heavier isotope, and calcium carbonate, relatively enriched. Estimates are at present that the burial rate of carbon in the carbonate sink is four times that in the organic carbon sink. The average ratio of the two carbon isotopes in the ocean at any time reflects the balance of these geological cycles. For example, an increased supply to the ocean of carbon from organic carbon weathering, all else being equal, would result in a lower ratio of ^{13}C relative to the lighter isotope in the ocean overall.

Surface-to-deep carbon isotope patterns can be studied by comparing the carbon isotope ratios in the calcite shells of surface and deep-dwelling organisms, as this material forms with little difference between its carbon isotope ratio and that of dissolved inorganic carbon. Long-term changes in carbon cycling affecting the ocean, such as decreases in organic carbon burial or increases in weathering, can be monitored by the ratio of the two carbon isotopes in shell material or in bulk carbonate sediments, reflecting the oceanic inventories of the two isotopes. Mass extinctions should result in dramatic decreases in primary productivity, and thus in carbon cycling within the ocean and relative to its sources and sinks[4]. The surface-to-deep gradient in ^{13}C to ^{12}C should be greatly reduced and the ratio of ^{13}C to ^{12}C in the ocean should decrease temporarily. The decreased surface-to-deep carbon isotope ratio has been previously noted for several Cretaceous/Tertiary boundary sequences, and a negative carbon shift has been observed for both of these extinction boundaries. The contributions[1-3] discussed in this article make clear the complexity of these transitions and the extended nature of the changes in oceanic carbon cycling, as recorded in carbon isotope records (see figure).

Holser *et al.*[1] present a detailed carbon isotope record from a continuous marine sequence in the Austrian Alps spanning the Permian/Triassic boundary, the most dramatic extinction event in the Phanerozoic. They conclude that this boundary was preceded by at least several million years of net oxidation of global reservoirs of organic carbon, and was followed by several anoxic episodes resulting from the long-term increase in the oxidation of organic carbon. The identification of these episodes of anoxia is supported by iron, sulphur and other minor-element data. But whether anoxia occurred globally or locally is unclear without data from other locations. The recovery to more typical carbon isotope values, and presumably to more normal oceanic oxidation–reduction conditions, took at least a few million years, indicating the complexity and long duration of this transition.

Gruszczyński *et al.*[2] use a carbon isotope record from brachiopod calcite from West Spitsbergen (present-day Arctic Ocean) to argue that the late Permian had a relatively brief interval of greatly increased organic-carbon burial before the start of the record presented by Holser *et al.* . The subsequent carbon isotope decline in their composite record is the largest yet observed in the late Permian. They suggest that the increased oxidation of organic carbon may have affected oceanic nutrient and atmospheric oxygen levels sufficiently to result in the mass extinctions.

Zachos *et al.*[3] conclude that the



Carbon isotope data and interpretations across the Permian/Triassic boundary discussed in refs 1 and 2 (ranges marked by hatching). The marine sequence discussed in ref. 3 shows four significant carbon-cycling changes in an interval that includes the Cretaceous/Tertiary boundary.

Cretaceous/Tertiary boundary was preceded by an interval of around 200,000 years of reduced net organic-carbon fluxes to the ocean. The boundary itself is marked by an extremely rapid cessation of oceanic primary productivity over less than 10,000 years, consistent with a bolide impact cause for these extinctions and apparently not related to the preceding shift in carbon isotopes. The drastic reduction in primary productivity persisted for at least half a million years at this low-latitude North Pacific site, with carbon isotope gradients between the surface and deep ocean not recovering to fully normal until about a million years after the boundary. This interpretation of these primary-productivity changes is enhanced by records of calcium carbonate accumulation and preservation and barium accumulation (as a proxy for organic carbon) at this site. Confirmation of the ocean-wide duration and intensity of these events requires more widespread geographic sampling of marine sequences spanning this boundary.

These three reports suggest intriguing links between carbon cycling and extinction events, with both events influenced by and resulting in changes in organic carbon cycling. Sea level seems to exert an important control on net rate of carbon oxidation and burial, with regressions favouring oxidation over burial[1,3]. Better knowledge of the relationship between sea level and carbon cycling, along with unambiguous records of sea level, is required to document the impact of sea level changes.

There is a limit to what records of oceanic carbon isotope composition, used so effectively in these reports, can reveal about mechanisms and results of extinction events. As discussed above, the distribution of carbon between carbonate carbon and relatively ^{13}C-depleted organic-carbon sedimentary reservoirs controls the average oceanic carbon isotope ratio, with the surface-to-deep gradient determined by organic-carbon formation and regeneration. Dissolved phosphate, a critical, limiting nutrient supplied to the oceans by continental weathering, is incorporated in organic matter in primary productivity and recycled back to the dissolved state by organic matter regeneration in deep waters. Surface waters have relatively low dissolved phosphate concentrations, therefore, and deep waters are phosphate-enriched.

Net organic-carbon burial in the sedimentary reservoir is an important sink for phosphate, estimated to account for 40 per cent of the total removal at present. Organic carbon is thus an important phosphorus carrier in the water column and an important removal mechanism for phosphorus from the oceans; any carbon cycling change has to affect oceanic phosphate inventories and distributions. For example, the marked decline in primary productivity inferred at the Cretaceous/Tertiary boundary should result in a more uniform water-column distribution of phosphate concentrations. The increased phosphate concentrations in surface waters, considered alone, should in turn result in increased rates of primary production, which would tend to re-establish the water column phosphate gradient.

Also, the increased weathering of organic carbon before the Permian/Triassic boundary probably would have resulted in increased phosphate inputs to the ocean. These increased phosphate inputs should result in higher rates of primary productivity in the water column, if oceanic turnover times were similar. This in turn could result in higher rates of organic-carbon burial, negating the effects on the carbon isotope composition of increased weathering. Obviously, the mechanisms relating phosphate inventories, carbon isotopes and primary productivity are more complex than these simple examples. Carbon-isotope signatures alone cannot reveal linked changes in oceanic nutrients and primary productivity. Geochemical tracers indicating nutrient concentrations and productivity variations are needed to unravel oceanic carbon cycling changes around extinctions; Zachos *et al.*[3] take a first step by using barium fluxes as indicative of organic-carbon fluxes. Ultimately, because organic-carbon weathering and burial influence atmospheric carbon dioxide and oxygen levels[4,5], an understanding of the links between these cycles will help to quantify atmospheric changes which can influence climatic changes. □

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RNA structure

Unwinding with a vengeance

Robert A. Lamb and Gideon Dreyfuss

UNTIL very recently, the old rule was that by examining the sequence of the genome template the RNA product could be predicted. The new rule is that the old rule is not quite good enough any more. In the past two years, a plethora of findings have underscored the tremendous plasticity of RNA molecules and the amazing variety of reactions they undergo. Recent examples include the two apolipoprotein B messenger RNAs, one of which has a uridine (U) residue instead of a templated cytidine (C)[1,2]; the extensive addition of non-templated U residues to kinetoplastid mitochondrial mRNA in trypanosomes[3]; and the addition of two non-templated guanosine (G) residues to a paramyxovirus mRNA[4]. Now, in the 23 December issue of *Cell*[5], Bass and Weintraub report a striking addition to this new collection of modifications that change RNA structure. These investigators have discovered that a previously known[6–8] ATP-dependent unwinding activity also covalently modifies double-stranded RNA substrates by converting adenosine (A) to inosine (I) residues. This modification weakens the capacity for base-pairing and brings about essentially irreversible separation of the strands.

Other double-stranded (ds) RNA unwinding activities, both ATP-independent (for example, ref. 8; and several pre-mRNA proteins, see below) and ATP-dependent (such as the eukaryotic elongation factor eIF-4A, ref. 9) have been described in many organisms, but so far none of them has been shown also to involve modification of the RNA. As such, this novel activity described by Bass and Weintraub[5] accomplishes the task with a vengeance, as the change to the structure and composition of RNA is both profound and irreversible.

The dsRNA unwinding/modifying activity was detected by careful but

serendipitous observations during studies on anti-sense RNA. What might be the normal purpose of this irreversible process? The most obvious answer might be an antiviral defence mechanism. All RNA viruses have to go through a dsRNA intermediate in their life cycle. Although it is well established that one general defence response triggered by the dsRNA form is the induction of interferon, the unwinding and irreversible modification of RNAs that are in a dsRNA form could complement the interferon effects. But does this in fact happen?

It is conceivable that the footprints of an antiviral dsRNA unwinding/modifying activity may have been left with unexpected and disastrous consequences, and may even have caused a human disease. Measles virus, a member of the paramyxovirus–morbillivirus family, has a negative-stranded RNA genome that is transcribed in the cytoplasm by the virion RNA transcriptase into a series of positive-strand mRNAs. In very rare cases of measles virus infection, a persistent infection occurs that is thought to involve defects in functions related to viral assembly, but not to RNA replication. This can lead to subacute sclerosing panencephalitis (SSPE), a progressive disease of the central nervous system.

Recently, Cattaneo *et al.*[10] reported nucleotide-sequencing studies on cloned mRNAs isolated from autopsy material of SSPE brain. In one case they found that a discrete measles virus mRNA, whose protein product (M protein) is essential for virus assembly, has more than 50 per cent of its U residues changed to C. Although biased hypermutation caused by infidelity of the viral RNA transcriptase while transcribing only the M gene cannot be excluded as a mechanism for this[10], it now seems plausible that the establishment of the persistent infection that led to SSPE results from the newly described dsRNA unwinding/modifying activity. If, during transcription of the viral genome, the M gene mRNA fails to leave the template, the dsRNA unwinding/modifying activity could change the A residues on the template RNA to I. In the next round of transcription of mRNA these I residues are copied to C residues. This two-step conversion mechanism could explain the extensive U to C changes found in the mRNA. It should be emphasized that this is not the sole explanation for persistent infections of measles virus. But the strong mutagenic potential of the dsRNA unwinding/modifying activity may have far-reaching consequences.

Another possible function of this novel activity could be its capacity to change specific RNA molecules, or groups of RNA molecules, including mRNAs. This could be accomplished by expression of complementary RNAs (such as small-nuclear RNAs) either to specific regions within introns of pre-mRNAs, which could lead to alternative splicing patterns, or by modifications within the exons to bring about changes in the coding specificity. For example, a complementary small RNA that base-pairs to a region of an intron of a pre-mRNA which specifies its splicing pattern could stimulate the dsRNA unwinding/modifying activity to alter the relevant sequence such that it alters its normal splicing pattern. It is also possible that the activity found so far is merely one of many such activities and that other related ones with greater specificity lead to changes such as that found in the apolipoprotein B mRNA[1,2]. Perhaps the activity is a general way of ensuring that segments of RNA that have the tendency to form double-stranded structures (intra- or inter-molecular) are maintained in single-stranded form. Should this not be reason for those in the field to look for inosine in their RNAs?

What could regulate dsRNA unwinding/modifying activity and prevent it from modifying most RNAs in the cell? Two general categories of regulation can be considered. First, there may be specific regulatory components that interact with it but which were separated from it during fractionation. Second, there probably exist activities that can counter the unwinding component of the activity, such as annealing–promoting proteins and proteins that block and protect regions of dsRNA from dissociation. A potent annealing activity has recently been described in nuclear extracts[11]. Furthermore, RNA molecules exist in cells as RNA–protein complexes with RNP proteins. The recent finding of a complex assortment of RNA-binding proteins in pre-mRNA RNP complexes is an illustration of the abundance and diversity of RNP proteins[12]. Many of them, being RNA-binding proteins[12], can function in unwinding and annealing of RNA molecules, and can bind to specific RNA sequences[13], perhaps protecting them, and thus decisively influence RNA structure. Taken together with the sequence of the RNA itself, the interplay among all these factors will determine the structure and thereby the function of RNA in the cell. □

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Daedalus

Fictitious fat

As many would-be slimmers know, the body has a preferred weight which it defends stubbornly. Even if forced to unload fat, it does so in a specific sequence, which (as in the case of the dreaded 'pear-shape syndrome') may not correspond to the desires of the slimmer. An adult body has a fixed number of fat cells; a successful slimmer succeeds in emptying some of them, while others nearby remain full. This, says Daedalus, makes good chemical sense. A droplet of fat in a cell is potentially metastable. If metabolic demand begins to shrink it a bit, its radius will decrease, its internal pressure will rise, its free-energy balance will shift towards further shrinkage, and the process will accelerate until the droplet is completely metabolized. So a fat cell can have only two stable states, full and empty. It is in fact a digital information-storage element. And this, says Daedalus, is how the body knows how heavy it is. Even within a fat deposit, the cells fill up in a preferred sequence, and it need only record the position of the currently active cell to know how far the process has got. This 'full up to here' signal is presumably relayed by the nerve connection from the cell.

This theory explains the digital accuracy with which some people maintain a steady weight despite their chaotic eating habits. It also suggests a new, powerful, and effortless way to lose weight. You merely generate a false 'full up to here' signal, suggesting to the body that a specific fat deposit is far fuller than it really is. It will then shed fat from that deposit, in an effort to restore the proper apparent loading.

So DREADCO volunteers are being gorged on a wildly calorific diet, and subsequently starved back to normal weight, while an NMR tomographic-imaging system maps the preferred sequence of filling and emptying of their fat cells. Daedalus will thus locate the last cell to be filled in each fat deposit. A false 'full up to here' signal from this cell would imply that the deposit is utterly full. So mild electric shocks at this exact location should induce rapid and effortless slimming. Indeed, a commercial shock-slimming system, claimed to work by stimulating the muscles, probably owes its sporadic success to this effect. But DREADCO's version will launch its false signals with reliable accuracy, giving far more dramatic results. Conversely, a false signal launched from the first-filled cell of a fat deposit will cause that deposit to gain weight.

Thus DREADCO's Programmed Electric Body Sculpture will reshape the user's fat deposits under full control. The pear-shape syndrome will be banished forever!

David Jones

Seal vaccination success

SIR—In providing evidence that the primary cause of the epizootic of distemper with high mortality among harbour seals (*Phoca vitulina*) in the North and Baltic seas was canine distemper virus (CDV) or a closely related morbillivirus[1], which has since been claimed to be a new morbillivirus christened phocine distemper virus (PDV)[2,3], we heralded our evaluation in seals of a subunit CDV vaccine, shown to be effective in dogs[4]. If seals can be protected by the vaccine, it would not only confirm the close relatedness between PDV and CDV, but it would also provide final proof for this morbillivirus as the primary cause of the recent epizootic in seals. We now report our initial success with two different vaccines.

We used animals belonging to a breeding group of harbour seals kept in isolation for many years. So far no signs of phocid distemper have been observed in this group. For ethical and practical reasons the study was limited to eight adult animals. Six of these were vaccinated twice intramuscularly at two-weekly intervals followed by a third vaccination four weeks later. Two received a candidate subunit iscom CDV vaccine, prepared in collaboration with Coopers Animal Health Ltd (Berkhamsted, UK) as previously described[4]; the other four were vaccinated with a candidate inactivated whole virus CDV vaccine, kindly provided by Duphar BV (Weesp, The Netherlands). The other two animals were sham-vaccinated with an antigen-free preparation.

Before vaccination, CDV-neutralizing serum antibodies were undetectable (titre below 10) in these animals. After the third vaccination all six CDV-vaccinated animals had antibody titres ranging from 300 to 1,000. Ten days later all eight animals were transferred to a closed swimming pool and challenged, intraperitoneally or oculo-nasally, with a suspension of organ material from seals that had died during the outbreak.

Given similarly, this material produced clinical disease and the development of CDV-neutralizing serum antibodies in dogs[5].

The two sham-vaccinated seals showed signs of acute respiratory disease and mucopurulent nasal discharge about two weeks after challenge and died on days 14 and 18. Viral antigen was detected in the spleens and/or lungs of both these animals with an enzyme-linked immunosorbent assay, using a monoclonal antibody which recognizes the F protein of both CDV and PDV[3-5]. No clinical signs were noted in any of the vaccinated animals (I. Visser, to be published). A threefold or higher (average 15-fold) increase in CDV-neutralizing serum antibody titres observed in all vaccinated animals two weeks after challenge suggests that some replication of PDV had taken place. As is often the case in the natural disease, CDV-neutralizing antibodies were not found in serum samples collected after the death of one of the two seals, probably because of the acute nature of the disease. The other had developed a CDV-neutralizing antibody titre of 30.

Although the number of animals studied is small, the data warrant the conclusion that seals can be protected from phocid distemper by vaccination with certain inactivated CDV vaccines. Since the disease was only produced in animals not vaccinated against canine distemper, the last of Koch's postulates has also been fulfilled, reconfirming that infection with CDV or a closely related morbillivirus is the primary cause of the epizootic.

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Superconducting single crystals

SIR—Were large single crystals of the high-temperature superconductors of the type $La_{2-x}A_xCuO_4$ available (where A represents Ba or Sr), their physical and structural properties might provide insight into the still unknown mechanism of high-temperature superconductivity. By use of the travelling-solvent floating-zone method[1], we have grown such crystals and determined their superconducting properties, which we report. The anisotropy of their magnetic and electrical properties will be described elsewhere.

Appropriate amounts of La_2O_3, CuO and $SrCO_3$ powders were mixed in ethanol, dried and calcined in air at 1123 K for 12 hours. The calcined powder was formed into a cylindrical shape, 6 mm in diameter by 50 mm in length, and compressed at a hydrostatic pressure of about 100 MPa. This rod was sintered at 1273–1473 K for 12 hours in oxygen gas and was then used as both the feed and the solvent rod. For crystal growth we used a double-ellipsoidal infrared heating furnace (Nichiden Machinery Ltd) heated by two 1.5-kW halogen lamps[1]. Growth conditions involved growth rates of 1.0–3.0 mm per hour, solvent compositions of 55–80 mol% CuO and a growth atmosphere of 0.2 MPa O_2 (to prevent vaporization of copper oxide from the melt zone).

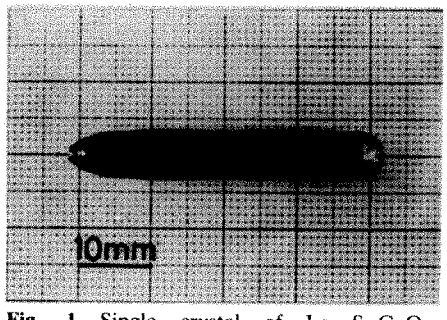


Fig. 1 Single crystal of $La_{2-x}Sr_xCuO_4$. Dimensions are ~ 6 mm diameter and 40 mm length.

A sample of $La_{2-x}Sr_xCuO_4$ in this way, using a solvent of 75 mol% CuO, is shown in Fig. 1. The black, lustreless region at one end comprises a two-phase mixture of La_2O_3 and $La_{2-x}Sr_xCuO_4$, but the remaining portion, black with a metallic lustre, is single-phase $La_{2-x}Sr_xCuO_4$. This region exhibits two facets parallel to the growth direction. Back-reflection Laue X-ray diffraction of these facets gives sharp diffraction spots, confirming the single-crystal nature, and reveals a fourfold rotation axis, identifying the crystallographic plane of the facets as (001). Although the samples contained some cracks, single crystals of about 6 mm in diameter by 15 mm in length could be obtained.

The lattice parameters of the tetragonal

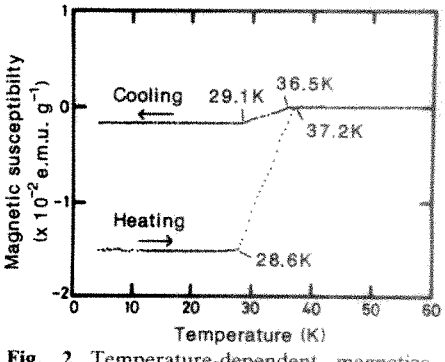


Fig. 2 Temperature-dependent magnetization of $La_{2-x}Sr_xCuO_4$ single crystals. The upper curve is for cooling in a field of 0.936 Oe; the lower curve is for heating in 0.936 Oe after cooling in zero field.

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$La_{2-x}Sr_xCuO_4$ crystals, measured by powder X-ray diffraction using Si as a standard, are $a=3.793\pm0.003$ Å and $c=13.20\pm0.02$ Å, which are consistent with values obtained from a sintered $La_{1.88}Sr_{0.12}CuO_4$ feed rod ($a=3.792\pm0.003$ Å, $c=13.21\pm0.02$ Å). Electron probe microanalysis indicates that the Sr content is uniform throughout the crystal, with $x=0.12$; for the feed material, $x=0.15$.

Figure 2 shows the magnetic susceptibility of $La_{1.88}Sr_{0.12}CuO_4$ as a function of temperature, measured using a SQUID magnetometer. The transition temperature observed on cooling the sample in a magnetic field of 0.936 Oe agrees well with that from heating the zero-field-cooled superconducting phase through the transition in the presence of the same field; but the magnetization differs considerably between these two cases. The transition onset temperature and the end temperature are 37 K and 29 K respectively, so that the width of the transition, ΔT_c, is 8 K. This transition is considerably sharper than both that for sintered $La_{2-x}Sr_xCuO_4$ (ref. 2), and that reported previously for a $La_{2-x}Sr_xCuO_4$ single crystal. We found a Meissner effect of 13%.

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Isotope dating of the Australian regolith

SIR—The work reported[1] by Bird and Chivas is based on three assumptions: (1) that materials recognized as forming deeply weathered profiles have been formed at some time in the past, on landscapes of low relief under tropical conditions; (2) that the date of such intense periods of weathering can be established by a method of sufficient accuracy to calibrate their oxygen isotope results; and (3) that the clay minerals used for oxygen-isotope analysis were formed during the intense weathering process.

We investigated the first assumption at two sites. The first site was the laterites and deeply weathered profiles on the Hawkesbury Sandstone near Sydney. These materials are generally regarded as erosional remnants of a fossil soil, formed in the Miocene and/or Pliocene, under intense tropical weathering, on a surface of low relief [2,3]. Our mineralogical, geochemical and palaeomagnetic investigations[4-6] demonstrate that these laterites and associated deep-weathered profiles were formed during the gradual stripping of the impervious cover of the Wianamatta shales, which provided the necessary environmental conditions for the mobilization of iron. In other words, all the laterites are the result of a time-transgressive process controlled by the rate at which the Wianamatta shale is being stripped from the Hawkesbury Sandstone, whereas the deep-weathering profiles are explicable in terms of normal bedrock variability.

Our second site was in the Cobar region of central western New South Wales, where silcretes and associated deep-weathered profiles had been recognized[7,8] as having developed on a Tertiary pediplain. Detailed mineralogical and geochemical investigations of these materials[6,9] show that the quartzitic Palaeozoic bedrock of these sites has remained essentially unaltered since a late Palaeozoic deformation and that both silcretes and deep-weathered profiles can be explained in terms of normal bedrock variability.

Thus, even though the materials at these two sites have all the characteristics of deep-weathering profiles and associated laterites and/or silcretes, there is no necessary connection between such characteristics and their formation under some past tropical conditions.

Taking their second assumption, Bird and Chivas themselves[1] doubt the application of stratigraphic and palaeomagnetic methods. Yet they used these methods to calibrate the oxygen-isotope results. They do not comment on how the difficulties were dealt with to make the calibration meaningful. For example, in commenting on the use of stratigraphic methods, Bird and Chivas refer to Exon *et al.*[10] to support the view that previous attemtps to date Australian regolith have often been unsatisfactory. That paper, however, is only the last of a series which throws light not only on dating problems, but also on definitional problems.

The use of palaeomagnetism as a method of dating seems to be similarly flawed, for our investigation of iron in the Hawkesbury Sandstone[5,6] suggests that no past intensive period of iron deposition coinciding with a period of deep weathering can be identified. What is indicated is that iron mobilization and deposition has occurred at a fairly uniform rate over the whole basin since the late Tertiary at least, and this is more than sufficient to explain any of the present-day iron accumulations. Our work casts doubt on studies that have established intense periods of iron deposition at some time in the Tertiary, and in particular on the determinations used by Bird and Chivas to establish the age of periods of deep weathering.

Finally, the long-term stability of the oxygen-isotope composition of kaolinites is fundamental to their use by Bird and Chivas in identifying the time of formation of clay minerals. If this is so, it can equally be used to support the contention that in any weathering mass there is inevitably a contribution from inherited clay minerals, which, as we have shown for the materials derived from the Triassic Hawkesbury Sandstone and the Palaeozoic rocks of Cobar, can be the dominant factor. Even though the degree of inheritance is less in profiles developed on igneous rocks, inherited material will always be present. Bird and Chivas do not appear to have considered this factor.

These doubts about the application of the methodology to the dating of deeply weathered regolith lead us to question the significance of the three ages of weathering identified by Bird and Chivas. Such weaknesses must be resolved before any further progress can be made in the study of deeply weathered Australian regolith.

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BIRD AND CHIVAS REPLY—We fully agree with Paton *et al.* that the Australian regolith is a spacially and temporally complex entity, and that weathering remains a major surficial process on the Australian continent. Many of the points raised are valid, indeed obvious; but stringent space requirements precluded full discussion[1]. We discuss these points in detail in a forthcoming paper[11], so here we will only correct some of the misconceptions held by Paton *et al.*

Their statement of the suppositions on which the study was based is erroneous. The supposition we make is that the isotope composition of meteoric waters in Australia has been changing with time.

We do not state that lateritization/deep-weathering requires tropical conditions and low-relief landscapes (although such conditions may aid the process); in fact, a major conclusion of this and our other studies[12] is that tropical conditions are not required. We do state that such weathering requires humid conditions and unimpeded drainage, and that as a result, infiltrating waters are not likely to have been subject to evaporative modification of their isotope composition.

We do not believe that such profiles can be independently dated with great accuracy (except in exceptional circumstances, such as dated weathered basalt overlain by basalt) which is why we have delineated only three age groups, spanning probably 200 million years.

We are aware that weathering can be an episodic or continual process, that weathering 'events' can be superimposed in a single profile, and that clay minerals can be inherited from the parent rock (although large quantities of kaolinite are

not common in most metamorphic or igneous rocks). But the increase in the isotope composition of meteoric waters with time means that low $\delta^{18}O$ clays (grossly out of equilibrium with modern waters) must have formed in ancient and cooler weathering regimes.

The division between fields B and C does not require the rejection of the independent age constraints, because in most cases the only independent constraint is simply that the weathering is older than mid-Tertiary. The division among pre-mid Tertiary samples is made on isotope grounds because the low $\delta^{18}O$ clays could not have formed during the comparatively warm climate of the late Mesozoic and early Tertiary in Australia. The isotope composition of the few samples of demonstrably pre-late Mesozoic age (such as kaolinite from regolith profiles beneath the Jurassic Wisanger Basalt, +10.2‰ and Jurassic Algebuckina Sandstone, +12.3‰) confirms the subdivision.

We are aware of the complexities involved in the palaeomagnetic dating of iron accumulations and, as indicated in our paper, we do not believe that there is necessarily a genetic or temporal link between the formation of a clay assemblage and an iron-rich zone in a regolith profile. We did not rely heavily on palaeomagnetically dated profiles for the reasons stated by Paton *et al.*, but in cases such as the Morney profile in south-west Queensland[13], the palaeomagnetic date can be taken as a minimum age for the development of the clay mineral assemblage.

With reference to the work of Paton *et al.* on the Cobar region, note that a kaolinitic sample collected from a roadcut 23 km south of Enngonia (which did not have any independent age control) yielded a $\delta^{18}O$ value of + 18.1‰ (post mid-Tertiary), consistent with the conclusions of those authors.

Finally, Paton *et al.* comment on the paucity of data points, particularly in the post-late Tertiary group, having previously described in detail the difficulties inherent in finding suitably unequivocal localities to sample. The post-late Tertiary samples we analysed were largely obtained from regolith developed on the late Tertiary–Quaternary volcanic provinces of Victoria and north Queensland. If Paton *et al.* wish to point out other such unequivocal localities, we would be glad to sample them.

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Origin of life

SIR—Miller and Bada[1] have made a valuable contribution to the understanding of the organic chemistry of high-temperature hydrothermal systems. They are, however, incorrect in citing my work[2] as supporting the hypothesis that life began in vents at mid-ocean ridges[3]. This important hypothesis has stimulated much debate[4] and is extremely interesting, but the evidence is strong[2,5,6], as Miller and Bada[1] have confirmed, that conditions are probably too extreme in submarine ridge vents.

The much more likely candidate for our birthplace is in subaerial (that is, land) hydrothermal systems. In them, temperatures at high level are commonly around 40 °C, with *p*H fluctuating, often even mildly alkaline. Closely juxtaposed parts of a system may vary greatly in temperature, and both water-dominated and relatively dry gas-dominated conditions are possible, often in rapid succession in the same place. There may be rapid influx of fluid of very different chemistry, from considerable depth. Many potential mineral catalysts occur. It is for these reasons that I concluded[2,5,6] that the likely setting of an RNA world would be in a subaerial, meteoric water hydrothermal system. Perhaps nowhere else in the Archaean world were conditions so favourable for that extraordinary event, the origin of life.

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MILLER AND BADA REPLY — Nisbet proposes that the origin of life took place in 40°C vent waters rather than 350°C vents. Russell *et al.*[2] have also claimed that lukewarm vent waters were the site of important prebiotic reactions. The vent waters that are referred to are apparently hydrothermal waters cooled by mixing with sea water or by loss of heat to rocks. Because the high-temperature conditions would decompose all the organic compounds, the vent waters mixed with sea water would have less organic material than the ocean as a whole.

It is not clear what is to be gained from this process over heating parts of the primitive ocean to 40°C on a beach or in a lagoon. An alternative is to heat the primitive sea water to 40°C, but not much higher, by circulating it through heated rocks. This apparently does not occur near submarine vents, but may take place near volcanoes (H. Craig, personal communication). Again there seems little to be gained by invoking this type of process. The chemical reactions taking place at 40°C are more rapid but still similar to those at 0°C, with a few exceptions (for example, template polymerizations may not work at 40°C because of the melting of the helix). Taking the heat of activation of a reaction as 20 kcal mol^{-1} and assuming that 1 part in 10^5 of the ocean is at 40°C, then 99.9% of the reaction would occur at 0°C and only 0.1% at 40°C.

Russell *et al.* refer to the reported detection of glycine in Red Sea hydrothermal brines[2] as evidence that a biotic synthesis occurs in mild hydrothermal vents. This glycine is a contaminant, however, which bleeds from the Chelex resin[3-5] employed in the analytical scheme. In addition, no other amino acids were reported[2], which would be usual for a prebiotic synthesis.

If lukewarm vent water, and not the ocean as a whole, came in contact with minerals that catalysed critical prebiotic reactions, as proposed by both Nisbet and Russell *et al.*, our argument could change. It is not feasible to evaluate this possibility until the minerals and reactions are named. It is to be noted that an important role in prebiotic chemistry for minerals and clays has so far not been demonstrated. As for the role of hydrophobic surfaces a primitive oil slick[5] may have been more important than the mineral surfaces suggested by Russell *et al.*

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Barnacle taxonomy

SIR—Crisp and Fogg[1] attack what they consider "mere changes of opinion" in establishing and changing names of taxa above the species level. To alleviate attendant inconveniences, at least among the barnacles, they offer a remedy of

compromise between the classification of Linnaeus, in which all species were lumped under *Lepas*, and a current system[2,3]; namely, return to Darwin's classification of 1851–54 (ref. 4) and limit formal appellation of subsequently recognized species–group taxa to the subgeneric level.

It is possible, for some taxonomic reason or another, that a current genus might be more appropriately recognized as a subgenus. But it does not take a monkey wrench thrown into the gears of an entire system, as Crisp and Fogg have proposed, to enact such changes; when fine tuning is necessary, it can be made on an individual basis. No precedent would be set for, after all, "the despised" subgeneric category is still in use among the barnacles. Therefore I would like to offer the following rejoinder.

Darwin's attempts to estimate relationships between taxa, and concomitantly their patterns of diversity, were clearly hindered by taxonomic inadequacies and perceptions prevailing at the time. Many biological species were lumped as single species and, concomitantly, many now well recognized genera were lumped as single genera. Of *Balanus concavus* he wrote[4], ". . .a good instance of the amount of variation which seems especially to occur in most of the species which have very extensive ranges." But this and the half dozen other 'variable' species he recognized have all turned out to represent several to numerous species[5–7]. Thus, with their diversity and relationships poorly understood, it is no wonder Darwin found barnacle biogeography of "no particular interest, for the species are not sufficiently numerous; and, what is still more adverse, the genera, with unimportant exceptions, range all over the world; so that there is no scale of difference, and it cannot be said that these two regions differ in their species".

Thus knowledge of much of the diversity and our perception of the biogeography of barnacles languished for the more than a century after Darwin, despite the results of numerous expeditions and collections from deep as well as shallow water, and the advances in our understanding of the species concept and ecology. It took major systematic revisions to reach the point where barnacles became biogeographically interesting, and then to where they began not only to corroborate but to elucidate general principles in marine biogeography[8–11].

What Crisp and Fogg fail to appreciate is that taxonomic recognition of the relationships between species and species groups, at and above the generic level, is necessary if evolutionary pathways and patterns are to be recognized. Continuing adjustments to such a system, usually enacted when knowledge has increased to the point where relationships are no longer being accurately portrayed, are inevitable inconveniences. If someone eventually comes up with a better method of handling such a database, fine, but in the meantime the remedy of compromise proposed by Crisp and Fogg[1] does not satisfy these prerequisites.

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Antigen presentation by B cells

SIR—The recent letter of Lassila *et al.*[1] clearly demonstrates that B cells cannot be the antigen-presenting cells that lead to priming of helper T cells. This contrasts with data from several laboratories[2–4] demonstrating that B cells are required for the priming of $CD4^+$, class II MHC-restricted T cells that proliferate in response to antigen as discussed by De Franco[5]. We would like to offer the following explanation for these apparently conflicting results.

CD4 T cells belong to two separate subsets that differ in their functions[6–9] and in their requirements for priming[2–4,8–10] and accessory factors[11]. One subset of CD4 T cells (helper T cells, or Th2) is effective in helping B cells make antibody, while the reciprocal subset of CD4 T cells (inflammatory T cells, Th1) is active in T-cell proliferation assays, cytolysis, macrophage activation, delayed type hypersensitivity and in fact will suppress B-cell responses[12,13].

Studies in B-cell-deficient mice indicate that B cells are required for the priming of the subset of CD4 T cells that proliferate in response to antigen[2–4], but are not required for the priming of the subset of CD4 T cells that help B cells secrete antibody[8–10]. The data also demonstrate that the B cell required for priming the proliferating CD4 T cell must be antigen-specific and MHC matched to the CD4 T cell[4].

By contrast, the clonal expansion of the Th2 subset requires IL-1[10,11], a molecule expressed abundantly on macrophage membranes but not on B cells or dendritic cells. Thus, the macrophage is almost certain to be the cell that presents antigen: self MHC complexes required for the priming of this CD4 T cell subset[8,9].

Given these facts, it is not surprising that in the experiment of Lassila *et al.* the subset of CD4 T cells that helps B cells could not be primed in the absence of syngeneic macrophages. However, this observation cannot be taken to mean that no priming of CD4 T cells has occurred. Had they measured priming of the CD4 T cell subset that participates in *in vitro* proliferative responses, one would expect such priming to have occurred. Likewise, the seemingly paradoxical observation that transgenic mice fail to make antibody responses if they have B-cell but not macrophage class II MHC expression[14] is probably due to the requirement for macrophages in helper T-cell priming. Finally, if Th1 tend to be suppressive of antibody production, the problem of avoiding the priming of helper T cells by B cell idiotypes, on which the Lasilla *et al.* letter is based, may be moot.

The paper of Lassila *et al.* is an elegant demonstration of the fact that B cells are ineffective at priming those CD4 T cells that can help B cells. It will, however, require further study to determine whether this inability of B cells to prime CD4 helper T cells applies to all CD4 T cell subsets. Unlike the virgin CD4 T cells of Lassila *et al.*, we are certainly turned on by the idea that macrophages are required only to activate the subset of CD4 T cells that is most effective at helping B cells make antibody, while B cells almost certainly can prime those CD4 T cells that mediate inflammatory responses such as cytotoxicity, macrophage activation, or delayed-type hypersensitivity, as well as suppression of antibody responses[9,12,13].

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of less than 500 words and five references. □

Improving on Darwin

Niles Eldredge

Evolutionary Processes and Metaphors. Edited by Mae-Wan Ho and Sidney W. Fox. *Wiley:1988. Pp.333. £35.95, $85.*

EVOLUTIONARY theory continues to be characterized by sharp dissent and often acrimonious debate. The 'synthesis' — that relatively tranquil period that began roughly in 1930 and ended in the mid-1960s — in retrospect seems anomalous. Since its birth in the 1850s, the subject has for the most part been a battleground; controversy is nothing new, but rather the normal state of affairs.

Many modern evolutionary biologists, myself among them, have proclaimed the dissension to be healthy. After all, the very essence of the scientific process entails questioning the received wisdom of the past. Yet at times the dialogue seems to transcend the simple search for a deeper, more accurate description of nature. Sometimes the theme is less one of correction and building upon a body of accumulated knowledge and more a clarion call to throw out the old — often with little clear development of what might take its place. The multi-authored contents of Ho and Fox's *Evolutionary Processes and Metaphors* tread a precarious path along the border between the bright search for a better understanding of evolution, and the darker quest to expunge modern biology of what are perceived as the evils built into Darwin's original ideas. The book's lowest moments come when darwinian thought is presented as at best an outmoded metaphor (shades of Jeremy Rifkin's *Algeny*) and at worst the personification of evil, as when competition rather than cooperation is seen to be central to a description of biological nature. This, and the repeated calls for a 'new paradigm', are the least prepossessing features of the book. How often one wishes that scientists would not take Thomas Kuhn so literally: no one wants to be guilty of doing mere 'normal science', so the search for new paradigms to overthrow the old becomes the norm.

But, for all its flaws, *Evolutionary Processes and Metaphors* is not just another exercise in Darwin-bashing — although those in whom the response is reflexive to defend reigning orthodoxy will have little difficulty finding enough absurdities and false characterizations of darwinian evolutionary biology to dismiss the book outright. If David Hull is right[1], and science (and indeed the entire world of ideas) proceeds in a fashion that basically mimics natural selection, more can be accomplished by dwelling on what seems to be promising in the book under review than complaining about the more flagrant red herrings it contains.

There are two basic sources of progress in scientific discourse: new data pertaining to phenomena previously unexplored; and conceptual clarification of phenomena previously considered, in some instances prompted by new data that reopen old issues. For example, several authors broach the topic of 'self-organization' in various biological systems, reflecting a resurgence of interest in this theme in evolutionary biology. And a number of the 15 essays touch on the recently re-opened issue of Weismann's 'barrier'. Received wisdom, stemming from Weismann, held the distinction between germ line and soma to be inviolate, thereby ignoring the otherwise well-established facts that germ line is generated afresh from somatic tissue in plants, and is hardly 'sequestered' to the extent conventionally supposed in many animal phyla[2]. The weismannian distinction of soma and germ line, with its asymmetric causal emphasis that the germ line determines the soma, but that what happens to the soma cannot affect the germ line, became the central dogma of molecular biology.

That somatic change can indeed become heritable naturally affects views on how the evolutionary process works. Thus I was particularly struck by Cullis's essay reviewing heritability of somatic mutations in flax, which poses the very real possibility that "higher plants have a genetically controlled variation generating system". Cullis takes pains not to claim that such induced variation arises as a response to organismic needs (a core neodarwinian postulate is that variation is generated without regard to organismic needs, that is, it is random with respect to selection). Yet in the very next essay, which is otherwise a balanced and useful account of the evolutionary implications of "new genetic mechanisms", Pollard asserts that the flax evidence indicates that germ-line DNA can develop "genetic variation *in response to the modified condition*" (emphasis mine) — thus, in nuance at least, going rather farther than Cullis in posing a more severe if apparently unsubstantiated challenge to orthodoxy.

Natural selection, perhaps unsurprisingly, provides much of the book's thematic continuity, as much in the guise of 'metaphor' as 'evolutionary process'. Several authors question the propriety of the very term 'selection', as (they claim) nothing in nature is actually doing the selecting. It is, according to Ho (as but one example), as if natural selection had merely been substituted for Paley's Divine Watchmaker: Darwin fashioned natural selection as a mechanistic, naturalistic substitution for a concept of a supernatural Creator in order to explain organismic design in nature. While this is so, complaining that selection is an inappropriate term because there is no selector ignores the parallel with *artificial* selection that Darwin had in mind. It was simply a terminological parallel that led to the choice of word. The critique does not speak to the real issue: is there anything to the notion of natural selection as evolutionary process?

Some of the essays in the book, notably those by Ho, come close to concluding 'no'; because natural selection is an outmoded metaphor, it seems we are to conclude that it is not a process that occurs in nature, at least to any extent that would be helpful in understanding evolutionary history. In trying to emphasize the positive contributions of this book, though, let us consider a related theme — 'holism' — that seems to hold more promise. Some contributors complain that traditional selection theory treats organisms in a passive manner: organisms are commonly portrayed as being at the mercy of their environment. More strongly, a number of them feel that the organism has (in theory) been inappropriately divorced from the environment when actually organisms and environment form a holistic unity. Thus development, and phylogenetic changes in developmental patterns, can be seen as arising from interaction between organism and environment in a dynamic fashion that is not fully taken into account in conventional views of the relation between environment, adult phenotype and development — views in which natural selection plays, of course, an important role.

This line, when developed well (as in Oyama's essay) is thought provoking. Yet too often the conceptual lumping together of ontologically separate entities leads to confusion rather than clarity. The best example may be natural selection itself. Darwin saw that organisms are both economic and reproductive 'machines' (itself a loaded metaphor; in their introductory essay, Ho and Fox proclaim emancipation from just this sort of 'mechanical' world view). How well organisms within a local population fare economically, in a world of finite resources where not all offspring can survive to reproduce, is bound to affect their relative reproductive success. Yet the term 'fitness', defined holistically simply as probability of reproductive success, overlooks

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RESEARCH

NUTRITIONAL ECOLOGY OF THE RUMINANT

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and obscures the various elements that may contribute to differential reproductive success. It is useful, in other words, to distinguish economic from reproductive activities within single organisms, the better to understand what causes affect reproductive success. Similarly, eliding the distinction between organism and (physical and biotic) environment threatens to obscure causal pathways, whether they be the role of environment in 'selection', or the effect organisms have on their 'environments'. No one denies that organisms and environments are intimately linked — no more than anyone denies that organisms are individuals, despite the usefulness of distinguishing their economic and reproductive functions when it comes to understanding the nature of natural selection.

Many essays in the book appear to be summaries of past work, in particular those devoted to the implications of phenomena newly apprehended. Especially at the molecular level, knowledge of such phenomena can only enrich our understanding of evolutionary process. The assumption that molecular mechanisms that bias genetic transmission somehow call into question the validity of other biasing mechanisms posed in the past — natural selection, say — is fortunately only vaguely hinted at in some contributions. The essays I found most appealing (those by Hailman and Gray on methodological issues in teasing apart process from results in evolutionary studies; by Gordon and by Bateson on evolutionary aspects of behaviour; by Cullis on variation; and by Oyama on development) propose links to the darwinian paradigm on which they quite naturally wish to improve. Theirs seems the better grasp of the past; and they write with the implicit realization that, rhetoric aside, improvement in the conceptual basis of any science usually involves keeping what's right, fixing what's not and adding the apparent implications of newly gained knowledge. □

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Highs and hopes

Roger Guillemin

Messengers of Paradise: Opiates and the Brain. By Charles F. Levinthal. *Anchor Press/Doubleday, New York:1988. Pp. 229. $16.95.*

COMING after Abrams's *The Milk of Paradise* (1962) and Wright's *Dark Paradise* (1982), we now have Levinthal's *Messengers of Paradise*. Levinthal's purpose in this non-technical little book is to recount the story of the discovery in 1975 and 1976 of the enkephalins and endorphins, molecules found in the human brain (and in fact that of all vertebrates) which have the biological activities of opiates, and having done so to tell us how our own endorphins are responsible for the bits of paradise that occasionally come our way throughout life.

Levinthal, a professor of psychology at Hofstra University on Long Island, New York, first provides a short and entertaining history of the use of opium from antiquity to these days of massive illicit use. But why should the human brain have highly specialized receptors for the opiate molecules in the sap of the poppy? Because — so went the hypothesis — opium molecules possibly resemble some substances that are made normally by the brain and are involved in its dealing with those very same phenomena — pain and mood — that are modified by opiates. The next step was to prove it.

In yet another among many attemps to emulate Jim Watson's *The Double Helix*, Levinthal goes on to tell the story of the search for these endogenous ligands of the opiate receptors, and their final characterization. But the difference between Watson's book, and all the others that have purported to describe the high adventure and competition involved in the making of scientific discoveries, is that Watson was centrally involved in the work that led to the proposal of a structure for DNA. He knew what he was talking about. His imitators have generally had only second- or even third-hand knowledge of what they have described, and the resulting books, although plausible to the lay public, have been deeply flawed.

Such is the problem with this part of *Messengers of Paradise*. I do not remember Levinthal's name in the literature dealing with the opioid peptides in the days when I was active in that field. Thus, the chapters that are meant to describe the intellectual process involved in the research, as well as the accompanying human drama, are unsatisfactory. Here readers may be easily entertained, but they will not become well-informed. This is not a work of scholarship. An accurate and complete historical study requires considerable time and devotion, and above all a scholarly sense of purpose in the endeavour (for a superb and rare example, see H. F. Judson's *The Eighth Day of Creation*, 1979).

So much for the messengers. What about paradise? Simple: paradise is a form of bliss, or vice versa, that is entered or glimpsed thanks to endorphins *à toutes les sauces*. Struggles over pain, social comfort, alienation and addiction, the quest for coherence and certainty, ecstasy, and finally creativity and laughter, are the subjects of a series of vignettes in which

everything is explained or proposed to be explained by too much, too little or just the right amounts of opioid peptides in our triune brains. After all, Somerset Maugham, in an exquisite essay on Zurbaran as a mystic painter (published in *The Vagrant Mood*, 1952), said plainly that mystical ecstasy, "generally the result of prayer and mortification", "may also arise through the influence of a drug, opium, for instance".

I have a lot of sympathy for some of the ideas and proposals described by Levinthal, and nothing would please me more than to be convinced that they are substantiated or even substantiable. There is little doubt that the opioid peptides are involved, somehow, in our homoeostatic handling of a variety of painful events. But even here I say *somehow*, as there are still many unanswered questions. For instance, I do not know of a definitive demonstration that acupuncture 'works' exclusively through the endorphin system. The 'proof' in such statements is usually that the expected effects are allegedly prevented by prior administration of naloxone, a powerful opiate antagonist. In the hands of critical investigators, such studies are difficult to perform and even more difficult to control. Moreover, we now know of effects of opioid peptides which are not naloxone-reversible.

A characteristic of many of these facile interpretations is the persistent confusion between the significance of blood levels of endorphins (which represent levels of endorphins of pituitary origin) and what happens or may happen in brain levels of endorphins, in one system of neurons or another. We have, so far, practically no way of knowing what is going on in such systems, even in laboratory experiments let alone the clinic. Intravenous injection of massive amounts of β-endorphin into animals or man has no effect whatsoever on measured functions of the central nervous system, because peptide molecules of this type do not penetrate the blood–brain barrier. Thus, relating the long distance runners' 'highs' to their blood levels of endorphins is meaningless.

Yes, the discovery of the chemical structure of the endogenous opioids found in our brains was a remarkable achievement. But it is still too early to know what to make of it, as basic knowledge or in daily life. Most of the original questions about the role(s) in health or disease of the enkephalins, the endorphins, and the other opioid peptides that were recognized later, still remain wide open. For all I know, some of Levinthal's enthusiasm — and, let's face it, that of most of us originally involved in these discoveries — may still be vindicated. □

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A growing sphere of knowledge

Ghillean T. Prance

Biodiversity. Edited by E. O. Wilson. *National Academy Press: 1988. Pp.521. Pbk $19.50. Distributed in Europe by Wiley, £16.80. To be published in hardback next month, £27.85, $32.50.*

UNTIL recently, 'biodiversity' — short for biological diversity — was a term used only by a few biologists. Today it is the subject of bills in the US Congress and of US Aid funding, and it was the central concern of a special meeting of the Pontifical Academy. Biodiversity has become the dominating theme of many scientific conferences, as it was at the August 1988 meeting of the American Institute of Biological Sciences held in Davis, California.

There is good reason for this rapid upturn of interest in the topic. In the past two years, alarming details have emerged of the rates of destruction of the tropical rainforest that harbours some 50 per cent of the world's biological diversity. Satellite photographs have shown that, in 1987, 204,000 km^2 of forest were burned in Brazil, and for 1988 the estimate is near to 250,000 km^2, much of the loss being of primary rainforest. A few years ago, environmentalists were called to task for suggesting that the annual worldwide total of rainforest destruction was 50 km^2. But the proven rate of destruction, the patent effect on climate and the number of species that are in danger of extinction have now combined to make biodiversity the centre of scientific and political attention.

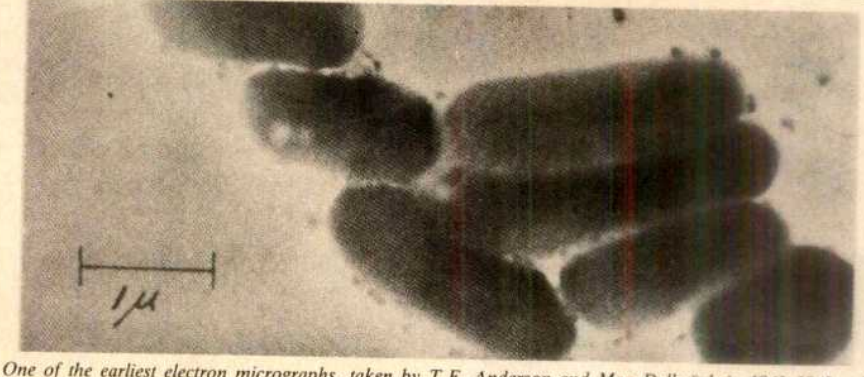
Biodiversity, the book under review, is based on a national forum which took place in September 1986 at the Smithsonian Institution, under the auspices of the US National Research Council. Made up of 57 chapters written by specialists, it is a thorough review of the many aspects of the subject. Although the emphasis is on tropical rainforest, the threats to many other ecosystems, such as grasslands, oceanic islands, coastal zones and oceans are covered. Two of the main facets of biodiversity are stressed — species diversity, and habitat and ecological diversity. Genetic diversity is not considered in any detail.

There is a particularly useful section of five chapters on the value of biodiversity, and another on the contribution science and technology can make. Here, the role of botanical gardens and zoos is given prominence. Other topics covered include agroforestry and other sustainable systems of management, political aspects of protecting diversity and ethical issues. The contents of the final section, "Ways of Seeing the Biosphere", vary from a native American account from New Mexico giving the viewpoint of a Pueblo Indian, to a description of the Earth as a living organism by James Lovelock, the creator of the Gaia hypothesis.

Each contribution is brief and to the point, the most of them have references to further work on the particular subject dealt with. The book does not contain many new ideas or concepts; rather, its value lies in bringing together an appropriate diversity of human talent to address a topic that is vital for the survival of life on Earth. It is written in such a way as to be understood by the general reader and, with its broad scientific content, will be used for teaching courses.

In the two years since the national forum was held, immense amounts of further evidence of the threat to biodiversity have been gathered. Yet with its comprehensive treatment the appearance of this book remains timely. It deserves wide notice. □

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One of the earliest electron micrographs, taken by T.F. Anderson and Max Delbrück in 1941–1942, of bacteriophage on the surface of E. coli. *It showed (amongst other things) that phage never enter the cell, an observation made earlier and independently by H. Ruska in Germany. The picture is reproduced from* Thinking about Science: Max Delbrück and the Origins of Molecular Biology, *by Ernst Peter Fischer and Carol Lipson (W.W. Norton, $19.95; to be published in Britain on 18 January, £13.95). The book is a translation and revision of Fischer's* Licht und Leben, *reviewed by Max Perutz in* Nature *320, 639 (1986).*

A walk on the wilder side

A. Rupert Hall

Let Newton Be! A New Perspective on His Life and Works. Edited by John Fauvel, Raymond Flood, Michael Shortland and Robin Wilson. *Oxford University Press: 1988. Pp.269. £17.50.*

Isaac Newton, an eccentric professor of mathematics at Cambridge unaccountably chosen for the chief office of the Royal Mint in 1696, was author of two books, published posthumously, *The Chronology of Ancient Kingdoms Amended* and the *Prophecies of Daniel and John*. Further, he left behind a vast quantity of manuscripts dealing with his business at the Mint, his views on the doctrine of the early Church, and alchemy. As scholar, theologian and monetary expert Newton was neither original nor up to date, and in these fields his work has been passed by without ever receiving much consideration.

If this had been Newton's obituary, it is doubtful that he would be of more interest today than Archbishop Ussher, even to scholars. But in fact Newton also wrote the *Principia*, *Opticks* and *Optical Lectures*, a large number of papers in *Philosophical Transactions* and several mathematical texts. It is a truism to affirm that if he has any place in the history of civilization, it is because of his scientific writings.

This elegantly produced collection of papers — with an appalling dust jacket — tells us more about the eccentric don than about the experimenter and mathematician known to scientists. It is all, or nearly all, quite true: Newton (like other mathematicians) did look into 'equal temperament' in music and he did have (to us) bizarre ideas about the reappearance of the harmonic ratios in other parts of physics, such as the optical spectrum (as Penelope Gouk relates). He did (as P.M. Rattansi and she report) draft learned pages on the Pythagoreans' ancient knowledge of the deeper principles of physics, such as the inverse-square law of gravitation. He did meticulously ascertain the life-span of the locust (species unstated) in order to calculate the prophesied length of the expansion of Islam. And he did (as J. Henry and J. Golinski explain) painstakingly copy out the (apparent?) gibberish of the alchemists, with their fanciful symbolic pictures, and himself experiment on the volatility of metallic salts and the reality of Diana's Doves.

As Keynes wrote long ago, Newton's mind was complex and strange. He accepted as unquestionable the priority of Hebrew learning. He was equally confident that ancient texts were pregnant with metaphors and codes, whose meaning could not appear to the unlearned. Only by discovering it, and so disclosing and restoring the pure wisdom of the infant world, could men perform their duty to God by understanding his Creation and Providence and so prepare for his Son's second advent. As P.M. Rattansi says, natural knowledge was but one element in Newton's *weltbild*, of whose theological character a strong hint is conveyed in the final scholium of the second *Principia* (1713).

Since Keynes (and so many others afterwards — nothing here is really new) read the pages that Newton would have kept hid, our knowledge of him has become richer but more confusing. Scholars are in danger of writing about the Newton who was insignificant to the neglect of the Newton who transformed thought. To be dismissive of Newton — as Whiteside, Westfall and Cohen, for example, have *not* been — is absurd. We see in this book two pictures of Harpo Marx representing Isaac Newton in the 1957 film *The Story of Mankind*, and many images taken from the books of the mystic 'platonist', Robert Fludd; it is doubtful if any of these is relevant to either Newton, the open or the concealed. Nor was Newton himself muddled about the natures of the diverse studies he pursued with equal ardour.

We need to be cautious about the inference that the scientific Newton drew inspiration and principles from the esoteric reading of his other self. My impression is that when experimenting or measuring or developing a structure of geometrical theorems, Newton was as much a 'scientist' as if he had had no other interests. In this book the investigations of the scientific Newton are well described, though necessarily in elementary language, by John Roche (*Principia*), C. Hakfoort (optics) and Jon Pepper (mathematics) — no place here for the kind of technical article ("Newton and the Calculus of Variations") that seemed appropriate 60 years ago. □

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Pollen stations

John Birks

Paleopalynology. By Alfred Traverse. *Unwin Hyman: 1988. Pp.600. Hbk £50, $75; pbk £24.95, $34.95.*

Palaeopalynology is the study of fossil pollen grains, spores and other organic microfossils (excluding diatoms and nannofossils) preserved in rocks and sediments. Among the objects of interest are acritarchs, dinoflagellates, microforaminiferal inner tests, chitinozoans, scolecodonts, selected algae, fungal, bryophyte and pteridophyte spores, and, of course, pollen from the recent past to the Precambrian. With such an enormous taxonomic diversity and stratigraphical range, it is inevitable that palaeopalynologists tend to be divided according to their specific stratigraphical or taxonomic interests, or both. And it is not surprising that, until now, nobody has written a book in English that sweeps across the subject as a whole.

Alfred Traverse, of Pennsylvania State University, has done just that. His book covers what palaeopalynology is and why it works; the basic biology and chemistry of palynomorphs; the stratigraphical palynology of the Precambrian and all the way through to the Quaternary; the production, dispersal and deposition of modern pollen; and applied palaeopalynology and laboratory techniques. It is profusely illustrated and has an extensive and up-to-date bibliography. Despite the enormous amount of information included, it is easy to read, with fascinating comments about many of the pioneers of the subject.

The book's greatest strength lies in the wealth of practical information it contains — the appendix on laboratory procedures is a monograph in itself and provides palynologists with an invaluable illustrated account of preparation techniques. The chapter on pollen morphology abounds in common sense and sound judgement, and includes a helpful synthesis of the different morphological terminologies and philosophies that are involved in understanding pollen-wall structure, along with an excellent account of the neglected topic of pollen orientation. The 30-page glossary is important for everyone concerned about precise terminology.

Traverse has maintained an admirable balance between the chapters on stratigraphical palynology and has avoided the temptation to put undue emphasis on the Tertiary (his main speciality). The book's weaknesses are the inevitable result of its breadth — some topics seem to my Quaternary palynological eyes to be covered too superficially. For example, there is virtually no mention of pollen-analytical studies of the effect of prehistoric man on vegetation, one of palynology's great success stories in Europe; and little is made of palynology's contribution to climatic reconstructions in the Quaternary or to modern plant ecology.

Traverse's book contains much of interest to all palaeopalynologists and it will, I hope, help to narrow the gaps between the different sub-disciplines. I urge all pollen analysts, novice or expert, to acquire and read it, and to share the author's manifest enthusiasm for his subject. □

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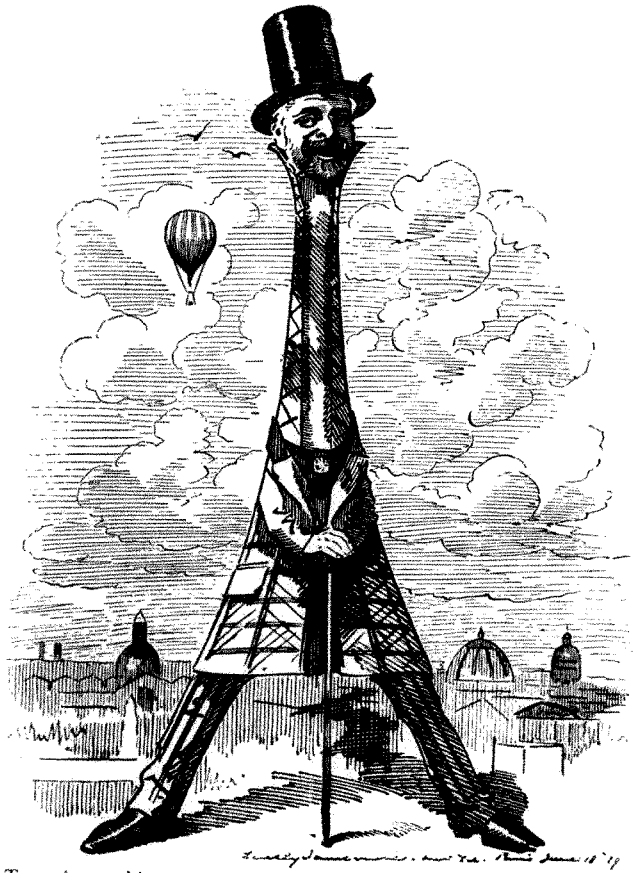
Eighteen eighty nine and all that

W. F. Bynum and J. L. Heilbron

An anniversarial cornucopia featuring rubber gloves, Louis Ilosvay de Nagy-Ilosva, the Eiffel Tower, nuclear fission, the Swedish Academy of Sciences, J. Willard Gibbs, the omega minus meson, Antoine Laurent Lavoisier, Timothy the tortoise and much, much more.

In continuance of the public service begun here last January, we again fill the slate of impending anniversaries according to the decimal system. In this we have subordinated our own preference, which runs to base 60, to the wishes of our many correspondents. The century remains our unit; boldface numbers in parentheses indicate multiples of a hundred years. The progress of science cannot be arrested for long, however. We observe, as a hint for the future, that 'hundred' has signified 106 in counting sheep, 120 in counting balks, boards and bomspars, 124 in counting cod or haberdine, and 225 in counting onions and garlic.

In the mundane reckoning, the French Revolution (**2.0**) may occupy pride of place. The storming of the Bastille on 14 July 1789 was greeted variously throughout Europe and the New World, but time has softened the passions and mellowed the emotions. We can hope that *liberté, égalité, fraternité* will be practised as well as celebrated in 1989. Science and technology, too, can claim their revolutions, both intellectual and social. Among the biggest of conceptual revolutions was that in chemistry, which flowered in the city of the Bastille in the year of its storming; Lavoisier, the prime mover in the one revolution, became a victim of the other. Among the social–technological revolutions on which we can reflect in 1989 are those founded on photography, nuclear fission, the jet engine and miracle drugs.



Towering achievement — a contemporary Punch *caricature of Alexandre Gustave Eiffel (Mary Evans Picture Library).*

1889 (1.0 centenary)

This year, in Spring, admirers of gigantism have to celebrate the hundredth anniversary of the completion of the Eiffel Tower. Built for the international exposition of 1889, the tower at 985 feet rose twice as tall as the tallest structure then in existence; it stood and stands for the height of wrought-iron technology. It has also been an instrument of scientific experimentation. In 1909 (**0.8**) Thomas Wulf designed a quartz-fibre electroscope especially suited to detect the presence of ionizing radiations. The following year he carried it to the top of the Eiffel Tower and found that its rate of discharge did not decline with height as expected. Confirmation and extension of this result during balloon flights by Victor Hess and others led to the discovery of cosmic rays. The underlying assumption of the measurement, that the leak of an insulated body exposed to the air does not in general arise from failure of the insulation, had been demonstrated definitively 20 years before (**1.0**) by C. V. Boys.

Shortly after the Eiffel Tower peaked out at 300 metres, Ludwig Mond — born 1839 (**1.5**), died 1909 (**0.8**) — found a cheap way to make replicas of it in nickel. This was the discovery of nickel carbonyl, which gave wings to the heavy metals (as Kelvin put it) and also much profit to Mond enterprises, because it led to a cheap way to extract nickel from poor ores. The profit in turn gave wings to British scientific and cultural life through Mond's benefactions, which included gifts to the National Gallery, the Royal Institution and the Royal Society.

Further towards higher things, 1889 was a fine year for students of the stars. Edward Charles Pickering (died 1919 — **0.7**), a passionate photographer of the heavens above Harvard, discovered the first known spectroscopic binary — a double star not resolvable by the telescope, but detectable by the periodic doubling of its spectral lines. Astronomers then residing on Mercury and facing the Sun would not have discovered this interesting phenomenon, nor, indeed, would they ever see any star but ours; for, as Giovanni Virginio Schiapparelli observed 100 years ago, Mercury constantly keeps the same surface towards the Sun. One of us insists on adding here that the total solar eclipse of New Year's day, 1889, was visible in California, and that a month later gratified observers founded the Astronomical Society of the Pacific. The total solar eclipse visible in Guiana just before Christmas does not seem to have inspired a similar organization.

With all this we have not mentioned the items of 1889 that showed science at its most promising, to one observer at least. In 1917, a Hungarian writer, Louis Ilosvay de Nagy-Ilosva, predicted a glorious development of science after the Great War. He rested his case for creative post-war scientific activity on a list of discoveries made after the Franco–Prussian War. For 1889 he gave the invention of organotherapy by Charles-Édouard Brown-Séquard (testicular injections which B-S believed had rejuvenated him); the demonstration by Nathan Zuntz that albumin, carbohydrates and fats are equally usable by the muscles; and the foundation by Albert I of the oceanographic museum of Monaco.

It might seem trivial to celebrate so humble a thing as the rubber glove, but modern surgery would not be the same without it. Rubber gloves had been possible since the vulcanization of rubber



National Museum of Photography, Film and Television

"Jabez Hogg and Mr Johnson", a daguerreotype taken in c. 1843. The picture is the earliest known of a photographer at work. Hogg is timing the exposure with his watch, holding the lens cap in his other hand; the head of Mr Johnson is supported by a rest, to help him to keep still for an exposure lasting as long as a minute. It is not known what part the caged bird had in the proceedings.

became practicable from 1839 (**1.5**); indeed various surgeons and accoucheurs had advocated their use thereafter, generally to protect themselves from nasty diseases from which their patients might be suffering. But the continuous history of the surgical glove dates from 1889, when W. S. Halsted asked the Goodyear Company to make two pairs, with gauntlets, for his scrub nurse, Miss Caroline Hampton (who married him the following year). Their original purpose was to protect Miss Hampton's hands from the disinfectant to which she was allergic, but the wearing of gloves gradually caught on for surgeons as well.

Halsted was the first professor of surgery at the Johns Hopkins Hospital, which opened in 1889 in anticipation of the Johns Hopkins School of Medicine (1893). Theirs will be a lengthy celebration.

No centennial list would be complete without mention of the transmigration of souls. In 1889 there died Michel Eugène Chevreul, aged 103, Warren de la Rue, James Prescott Joule and Maria Mitchell. The same year saw the births of Sewell Wright (who lived almost to witness his own centennial), Lord Adrian, Dirk Coster, Ralph Fowler, Beno Gutenberg, Edwin Hubble, Ludwig Wittgenstein and the historian of science Hélène Metzger. We have not been able to discover with certainty which souls went where.

1839 (1.5 centenary)

Let us hope that the productive mixture of science and art that gave us photography recurs in celebrations of the sesquicentennial of its invention. In August of 1839 Louis Jacques Mandé Daguerre — born 1789 (**2.0**) — painter of panoramas, proprietor of the Paris and London Dioramas, and careful chemist, announced his process for sensitizing metal plates, exposing them to light and fixing the latent image. The same year, William Henry Fox Talbot, Cambridge wrangler, philologist and frustrated landscape painter, made known his process for taking negative images on treated paper. Many far-reaching improvements perfected in the next half-century made photography an indispensable tool of science. By 1889 (**1.0**) the physiologist Étienne-Jules Marey could determine with its aid that a fly in flight flaps its wings 288 times a second, a busy bee only 190 times, a lethargic butterfly 9 times. And the physicist Friedrich Neeson could propose to investigate the details of a more sinister sort of flight, the speed and spin of artillery shells.

American physicists will want to do honour to their first great theorist, J. Willard Gibbs, born 11 February 1839 in New Haven, Connecticut, where he spent all his life after returning in 1869 (**1.2**) from three years of study in Europe. He made himself known by sending his precise and difficult memoirs on thermodynamics and statistical mechanics to the leading European physical scientists. Maxwell, Rayleigh and Ostwald read and admired: Helmholtz, Planck and Einstein read not, and had to recover Gibbs's results independently, and with difficulty. Gibbs's years in Europe overlapped those of his exact contemporary, Aleksandr Grigorievich Stoletov, who became professor of physics at the University of Moscow. His best-known work, which established certain regularities of the then-new photoelectric effect, is eligible for centennial observance.

Prophetic observers in 1839 might have read into the official opening of the Physiological Institute at the University of Breslau something of moment. At the least, they would have known that it was the first physiological institute within the burgeoning German university system and that its director and leading light, Jan Evangelista Purkyně (died 1869, **1.2**), had already made a number of important physiological and histological discoveries. Like any serious microscopist of his day, Purkyně had availed himself of the improved accuracy of the achromatic microscope: "With boundless eagerness I investigated within the shortest time all areas of plant and animal histology and concluded that this new field was inexhaustible".

After 1839, Purkyně could make use of a systematic theory of cells, enunciated in that year by Theodor Schwann, whose *Mikroskopische Untersuchungen* capped what had been five amazing years of experimental productivity. Schwann's cell theory is not our cell theory, for he postulated that these basic units of living organisms can be formed by a process analogous to crystallization from a non-living soup he called the *blastema*. *Omnis cellula e cellula* came a little bit later, and not from Schwann. He himself returned to the Roman Catholicism of his fathers, discovered the "God of the heart, not of reason", and sought enlightenment through mystical insights into the nature of things. Appropriately the Microscopical Society of London (now graced with Royal blessing) was founded in that year of microscopical revelation.

Geologists will want to note the death, on 28 August 1839, of William Smith, the surveyor and stratigrapher whose maps did so much to establish historical geology and palaeontology. Like virtually all geologists in the discipline's heroic age, Smith travelled indefatigably, although confining himself to the British Isles. Sir Roderick Impey Murchison's *Silurian System* of the same year limited itself to Wales and the west of England, although Murchison possessively investigated Silurian remains in Russia and elsewhere. Another travelling naturalist, Charles Darwin, also burst upon the Victorian reading public with his *Journal of Researches*, though with little hint of the ideas that were later to revolutionize the study of the natural world.

Our preoccupation with the health of the ozone layer may promote a sesquicentennial for Christian Friedrich Schönbein — born 1799 (**1.9**) — who in 1839 identified the origin of the odour around electrostatic machines and electrolytic oxygen as a new sort of gas. He

was then professor of physics and chemistry at the University of Basel, where he arrived following a curriculum vitae that included a strong dose of *Naturphilosophie* and an even stronger antidote, in the form of a year's stint as mathematics master at a boys' school in Epsom, Surrey. It took over a decade for Schönbein's colleagues to convince him that ozone is allotropic oxygen, and not, as he insisted, a chemical compound. As our next entry further illustrates, oxygen has not been an easy concept for its discoverers.

1789 (2.0 centenary)

Enlightened people wanted not only to talk well but also to talk correctly. French chemists shared and profited from this compulsion. In 1789 Antoine Laurent Lavoisier gave them a *Traité élémentaire de chimie* incorporating a nomenclature that was, in the buzz words of the Enlightenment, reasonable, natural and systematic. The revolution worked by his systematic vocabulary and the rules for applying it may best be illustrated by the substitution of words understandable by a modern chemist for terms now unintelligible, such as flowers of zinc and butter of arsenic. But just as even the most punctilious lexicographer sometimes fumbles etymologies, so Lavoisier invented some words that have proved unsuitable. Of these, 'oxygen', coined in the belief that dephlogisticated air is the principle of all acids, is perhaps the most interesting. Although Humphry Davy proved the etymology false in 1809–1810 (**1.8**) by demonstrating that muriatic acid (HCl) contains no oxygen, the misnomer survives. No one now has any difficulty with it; it is not necessary to know what a word means to use it correctly.

Another triumph of the natural and the systematic in 1789 was the publication of Antoine-Laurent de Jussieu's *Genera plantarum*, which presented botanists with an improved taxonomic system developed by the inbreeding of the ideas of three generations of Jussieus. This classification of flowering plants rested on correlation of a wide variety of characters and consideration of a very large number of specimens. Like Lavoisier's *Traité élémentaire*, Jussieu's *Genera plantarum* quickly became the cicerone in its field; although it has suffered many changes in its lower taxa, its arrangement of families has remained a part of botanical classification.

Jussieu was part of the increasingly professional tradition of French science, the Reverend Gilbert White a quintessential part of the amateur one in Britain. His book, *The Natural History and Antiquities of Selborne, in the County of Southampton* (1789), was a triumph of the local and parochial, couched with insight into the universal. Like Jane Austen, White lived through stirring times but mostly kept quiet about them. His observations on plants, birds, bats and his pet tortoise Timothy (who recognized the hand that fed him) continue to entertain, amuse and instruct.

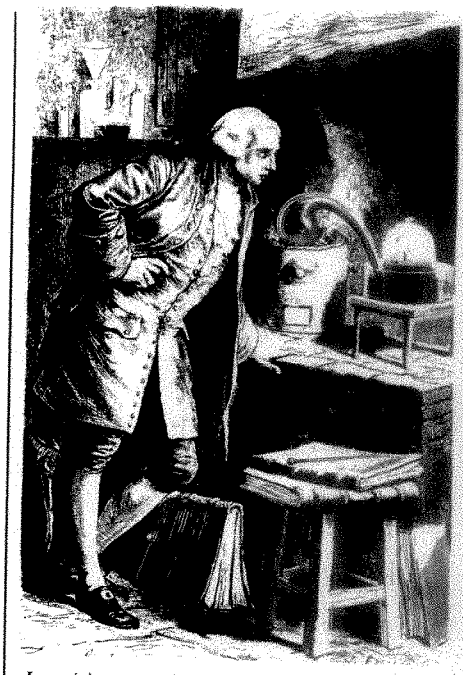
The year 1789 was a good one for the production of scientists named Bérard. Jacques Étienne Bérard, who died in 1869 (and so achieved a **2.0** and a **1.2**), is best known for his work with François Delaroche on the specific heats of gases at constant pressure. He later won a prize for discovering the effects of various gases on delaying the ripening of fruit. Joseph Frédéric Bérard, a physician, worked more in the classificatory and clarifying line. He began his short career with a *Plan d'une médecine naturelle*, and dedicated the rest of it to purging medicine of the misuse of words.

The greatest of French scientists born in 1789 was Augustin-Louis Cauchy, who gave the first comprehensive theory of complex numbers. His numbers were not his only complex. A little schizophrenia appears in his carrying to his first job, as engineer on the Napoleonic naval base at Cherbourg, Laplace's *Mécanique céleste* ("Sire, I have no use for that hypothesis") and Thomas à Kempis's *Imitatio christi*. In 1830 he chose religion over mathematics, refused the oath to the July monarchy, left his professorships and fled to the Jesuits in Fribourg. He returned to Paris in 1839 (**1.5**) and continued to pour out papers on all mathematical subjects; in less than 20 years no fewer than 589 (**14.0**) were published in the *Comptes Rendus* of the Paris Académie des Sciences.

1739 (2.5 centenary)

The year 1739 saw the foundation of the Swedish Academy of Sciences. It resembled the British, rather than the Continental, form of such societies in receiving from its king little more than the right to call itself royal. It emphasized practical arts as strongly as basic science. Among its earliest distinguished members were Anders Celsius, of the fixed-point centigrade thermometer (0° = temperature of boiling water and 100° = that of melting ice, a scheme that was later turned on its head); Carl Linnaeus, of the rigid-sex system of botanical classification; Mårten Triewald, who had learned his science and technology in England; and Emmanuel Swedenborg, who got his from angels. The Academy is now best known as the awarder of the Nobel prizes in physics and chemistry, and of the prize in Nobel's name in economics. In discharging this onerous responsibility, it can draw on 250 years' experience in balancing the pure and the applied, and on advice ranging from the angelic to the all-too-human.

The applied scientist might also celebrate 1739 for the publication of the second and last volume of Bernard Forest de Bélidor's *Architecture hydraulique*. This work rationalized civil engineering, in which shipbuilding and waterways then played a prominent part, with exemplary constructions and elementary arithmetic. Bélidor had previously written *La science des ingénieurs* — 1729 (**2.6**) — which deals almost exclusively with fortifications. It indicates an advance in professionalization, and also perhaps in civilization, that 'engineering' is no longer synonymous with 'military architecture'.

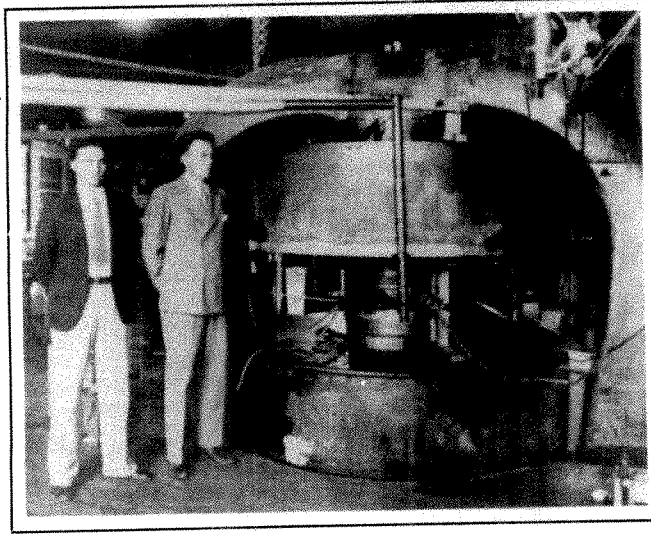


Lavoisier — prime mover in one revolution, victim of another.

1689 (3.0 centenary)

One of the greatest of all describers of disease, Thomas Sydenham (the 'English Hippocrates'), died on 29 December 1689. His happiest days had been spent as a young cavalry officer on the side of Parliament during the English Civil War, before settling down to become the leading London physician of his day. He described his own gout with especial feeling, but his portraits of smallpox, measles, hysteria and fevers mark him as a kind of Gilbert White of medicine. He brought an element of common sense into therapeutics, popularized the use of Peruvian Bark (which contains quinine) for intermittent fevers (the ague), invented a liquid preparation of opium (laudanum) and praised the value of fresh air and exercise. He had friends (including Robert Boyle and John Locke), but was not a clubbable man; above all, he believed in his own senses, which meant he had little truck with microscopes and other newfangled aids.

Consequently, Sydenham had no appreciation of the work of the Dutch lens grinder, Antoni van Leeuwenhoek, whose activity of 1689 included a study of the eye and the identification of the rods of the retina: seeing how we see. ▶



Lawrence Berkeley Laboratory

Man and machine — Ernest Orlando Lawrence (right) and the 37-inch cyclotron at the University of California, Berkeley, in 1936. This device was one of several of increasing power that Lawrence developed during the 1930s; his inventiveness was rewarded with the Nobel prize in physics for 1939.

1589 (4.0 centenary)

Armchair explorers should bestir themselves to celebrate the four hundredth anniversary of two books that immensely enlarged the knowledge and misinformation of the inquisitive reader of the late sixteenth century. One, Richard Hakluyt's *The principall navigations, voiages and discoveries of the English nation*, amounted to a handbook of the discoveries of the pushy navigators of Elizabethan England. Hakluyt understood the importance of acquainting his countrymen with the elements of geography; and for all the errors of his maps and reports, his readers knew more about the lie of the land than do most British and American high-school students, who cannot find London on a map or name the northern neighbour of the United States. The second mind-expanding book of 1589, Giambattista della Porta's *Magiae naturalis*, is a hodge-podge of recipes, parlour tricks, gee-whiz experiments and natural-philosophical claptrap. "Whatsoever was notable", says he, "and to be desired through the world, for curiosities and excellent things, I have abundantly found out"; which is the same principle on which this catalogue of anniversaries has been compiled.

1939 (0.5 centenary)

The fireworks have already started for the celebration of the fiftieth anniversary of the discovery of nuclear fission. As noticed in this column last year, Otto Hahn — born 1879 (**1.1**) — and Fritz Strassmann made and interpreted the critical radiochemical observations in December 1938. In February 1939, Lise Meitner and Otto Frisch — died 1979 (**0.1**) — published their idea of the physics at work, and appropriated the biological term 'fission'. Immediately physicists and journalists talked up the possibility of the practical application of atomic energy. Would it be Utopia or Armageddon? Also in February, Niels Bohr published his suggestion that the rare lighter isotope of uranium, uranium-235, was the source of the fission. It therefore appeared that the exploitation of the atom required the separation of heavy isotopes in bulk. Doubt about the possibility of effecting this separation persisted through the year, encouraging those who feared a bomb and frustrating those who hoped for power.

The Nobel prize in physics given during the bicentennial year of the Royal Swedish Academy of Sciences went to Ernest O. Lawrence for his invention of the cyclotron. By distinguishing the inventor of a machine and a method, rather than an investigator who made discoveries, the prize certified the arrival and the importance of the big, interdisciplinary, academic physics laboratory. The 1948 Nobel laureate for physiology or medicine was a chemist who made his observations in 1939. Paul Müller — born 1899 (**0.9**) — culminated his search for the perfect insecticide with the discovery that 4,4'-dichloro-diphenyl-trichloroethane satisfies many of the criteria. The patent on DDT, as he called it, was secured in 1940 and by the end of the Second World War the miracle chemical was available to help with programmes of insect control and disease eradication. The problems did not emerge until later. Other harbingers of the post-war world also appeared: in April 1939, RCA put its first television sets on the market; in August the German aircraft maker Heinkel flew the first jet aeroplane, too late to be operational for the Battle of Britain; and in December, a company incorporated to exploit Edwin H. Armstrong's patents for frequency-modulated radio, in good time to fight with RCA over the control of frequency bands.

It was already abundantly clear by 1939 that the war to end all wars had failed. Among those who did not live to see the horror of the coming years were Sigmund Freud, Harvey Cushing, Karl Friedrich von Auwers, George Barger, Frank Dyson, Søren Sørensen, Floyd Richtmyer and E. B. Wilson.

1914 and 1964 (0.5 ± 0.25 centenaries)

The celebrated experiments of James Franck — died 1964 (**0.25**) — and Heinrich Hertz, in which they measured the excitation potential of mercury vapour, took place in 1914. The fact that they at first misinterpreted their results as ionization potentials and hence as fatal to Bohr's new theory of the atom is less celebrated. In 1926 they received the Nobel prize "for their discovery of the laws governing the impact of an electron upon an atom", that is, for confirming Bohr's ideas. The detection of the omega minus meson in 1964 represents an entirely different sort of discovery, one driven by a theoretical prediction, in this case the system of quarks which won the Nobel prize for 1969 (**0.20**) for its inventor, Murray Gell-Mann — born 1929 (**0.60**). The discovery of CP violation, which also took place in 1964, showed that nature had not yet run out of surprises.

The Japanese contribution to modern science has been substantial. Many of the earliest pioneers who sailed from the Land of the Rising Sun to Europe went to learn the latest bacteriological techniques. Among them were Sahachiro Hata, Hideyo Nogouchi and Shibasaburo Kitasato. The latter worked with both Koch and von Behring and was the founder, in 1914, of the Kitasato Institute in Tokyo. On Christmas Day of the same year, in far-off Rochester, Minnesota, E. C. Kendall crystallized a substance which he called thyroxin. Crude thyroid extract was already used therapeutically but Kendall's achievement was to make possible the synthesis and elucidation of the molecular structure of the active thyroid substance. Kendall shared the Nobel prize in 1950: not for this research, important though it was, but for his work, with Philip Hench, on the isolation and therapeutic uses of 'Compound E' (cortisone), reported by them in 1949 (**0.4**).

We end, as we began, with the gargantuan. In 1914 the Panama Canal admitted its first ships after a decade of digging. Its completion required the conquest of yellow fever and the removal of 240 million cubic yards of earth. In 1964, the Verrazano Narrows Bridge, begun in 1959 (**0.3**), the longest single-suspension span in the world, opened for traffic. Each of its towers contains steel enough to make 18,000 automobiles, big ones, 1964-vintage; the entire structure weighs almost four times more than the Empire State Building. The atomic bomb exploded that year by the Chinese — their first — would have destroyed both the building and the bridge, had it been tested in Manhattan. □

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Chemical codes for the control of behaviour in arthropods

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Neuromodulators and hormones elicit and modify well-defined behaviours. Their mode of action can be studied to advantage in arthropods, where the natural releasing cells and neuronal target circuits are concisely identified. The coordinated actions of biogenic amines and peptides on both central and peripheral neural activity and metabolic processes bias the whole organism to perform a coherent behavioural routine.

THE Greek physician Galen believed that the brain, with its fluid-filled cavities, was mainly a secretory organ that regulated all aspects of health, mood and nervous function. The modern view of the brain stresses that its computational tasks are based on the connections between its constituent nerve cells. But chemistry plays a vital role in expressing this connectivity, the extent of which is only just being appreciated. Although the importance of chemical transmitters for mediating information transfer at the synaptic connections between neurons is well established, in the past few years it has become apparent that this transmission can be altered or modulated by other chemicals, also produced by neurons, known as neuromodulators.

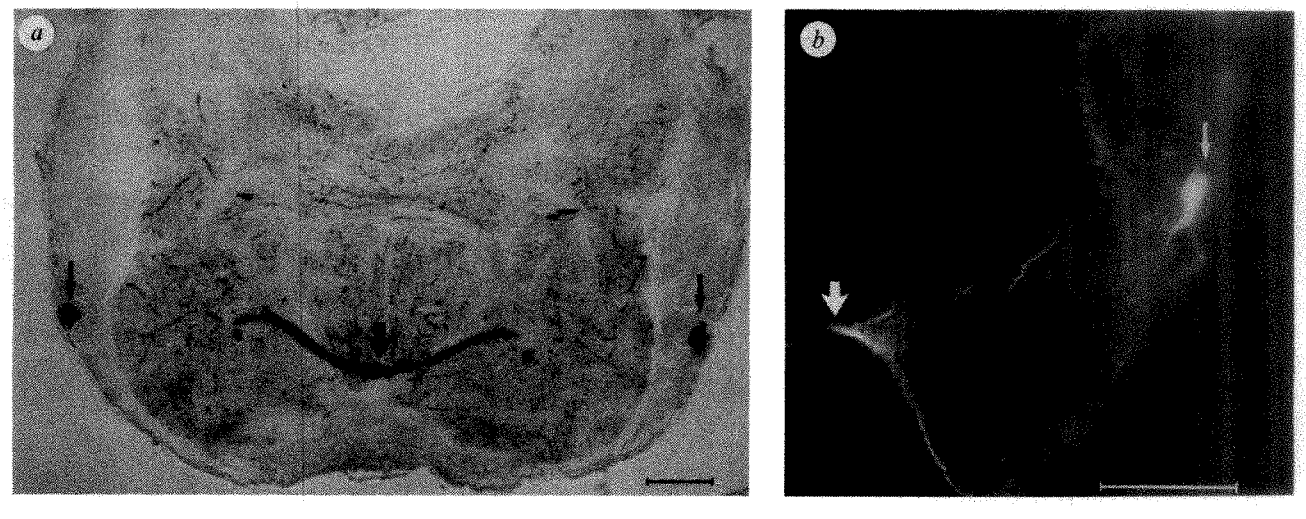
The actions of neuromodulators at the cellular level and their effects on behaviour are known in some detail but it has been difficult to bridge the gap between these two levels. Here we review recent work on crustaceans and insects which is demonstrating how modulators affect the connectivity in neural circuits, tuning and orchestrating their outputs to fit the immediate needs of the animal.

The production of behavioural sequences requires the temporal and spatial coordination of many neural circuits. In addition, there has to be flexibility in the ways in which circuits can be combined because sensory, central and motor neurons can participate in various combinations in many different behaviour patterns. The work in crustaceans and insects is showing that neuromodulators promote this plasticity by concordantly altering the responsiveness of neurons and muscles, together with changes in metabolism. Through the interplay of several modulators, basic circuitry can be allocated in a variety of ways at different times.

Transmitters, modulators and hormones

The 'classical' transmitters such as acetylcholine, glutamate and GABA directly affect the operation of ion channels and thus rapidly either excite or inhibit the postsynaptic cell. In contrast, the neuromodulators, which include biogenic amines and peptides, act indirectly on the ion channels, modifying their responses to the classical transmitters on a longer time scale. The neurohormones, a third class of chemical messengers, work in a similar way to the modulators, but usually over yet longer times and on targets at a distance from their release sites.

In mammals, neuromodulators and neurohormones are involved in generating specific behavioural states; experimental alteration of neuromodulator levels can evoke specific body movements, postures and motivational states, and alter sensory processing. For example, depletion of the biogenic amine 5-hydroxytryptamine (5-HT, or serotonin) in the brain leads to an increase in arousal and motor activity, evidence that 5-HT normally has a depressing or braking effect on behaviour[1]. On the other hand, stimulation of pathways that release another biogenic amine, noradrenaline, increases activity and may



Neurons in the insect nervous system can be identified by a combination of immunohistochemistry and intracellular injection of a fluorescent dye marker. *a*, Serially homologous neurons in the suboesophageal ganglion of the bee that react with antibodies to 5-HT (peroxidase-antiperoxidase reaction). *b*, One of these neurons after intracellular injection with Lucifer yellow. Subsequent processing of this preparation with antibodies to 5-HT confirms that this is one of the neurons shown in *a*. These neurons may be involved in modulating the proboscis-extension reflex, discussed on page 37. Scale bar, 50 μm. Small arrows: cell bodies; broad arrows: commissural fibres. Photos: Martin Hammer.

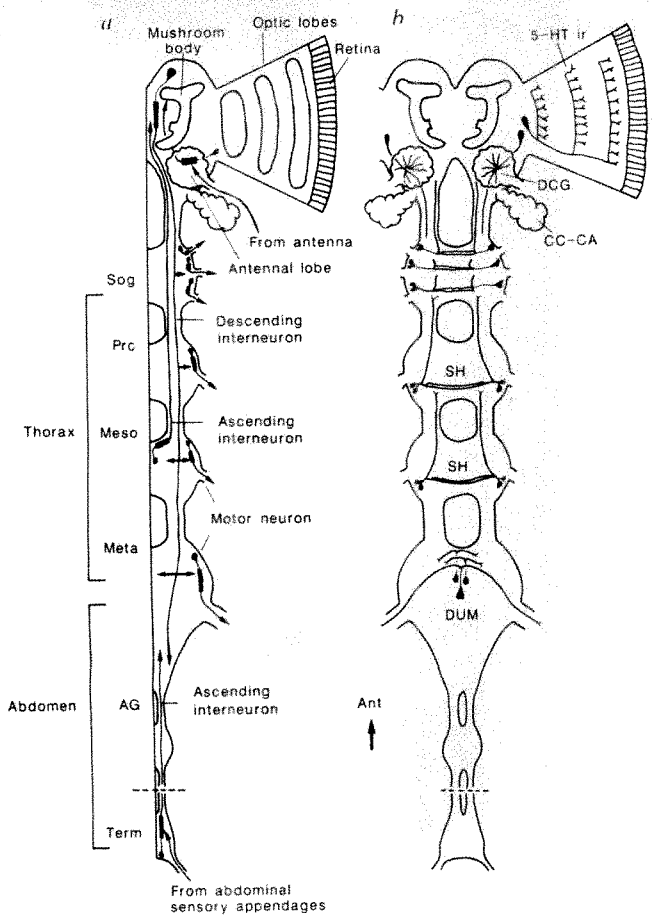


Fig. 1 Schematic drawing showing the organization of the segmented insect nervous system (*a*) and some pathways for information flow (*a* and *b*). The insect nervous system serves as a model of the arthropod nervous system, which consists of an anterior cerebrum (brain) and a ventral chain of segmented ganglia joined by paired connectives. The brain processes sensory information from the retina of the compound eye in the optic lobes and from sensory receptors on the antenna in the antennal lobe. Multimodal integration takes place in more central neuropiles, for example, the mushroom bodies, the output of which is transmitted to the motor centres of the ventral nerve cord by descending interneurons. The brain is linked by paired connectives to the suboesophageal ganglion (Sog) and connects to the hormone-producing neurohaemal organs, the corpora cardiaca (CC) and corpora allata (CA). The suboesophageal ganglion contains the motor circuits for feeding and collaborates with the brain in the initiation, maintenance and coordination of behaviours[71]. The ganglia of the ventral nerve cord contain the neural circuitry controlling behaviours such as flight, jumping, respiration, stridulation and oviposition, including the motor neurons that form the outputs to the muscles. These ganglia communicate with the brain through ascending neurons, so that the flow of information in the segmented nervous system is organized in a loop-like fashion[71]. Some identified classes of monoaminergic neurons interconnecting several neuropilar compartments, or even segments, are shown schematically in *b*. These include several 5-HT-immunoreactive neurons (5-HT-ir) in the optic lobes[72], a descending interneuron in the antennal lobe (DCG)[68] and segmentally homologous neurons (SH)[68,73]; and a cluster of octopamine-containing unpaired median DUM neurons[27-30] in each of the thoracic ganglia. Pro: prothoracic ganglion; Meso: mesothoracic ganglion; Meta: metathoracic ganglion; AG; abdominal ganglion: Term: terminal ganglion. Arrows indicate synaptic output areas; thickening of neurites indicates areas of synaptic input. Ant, anterior.

facilitate learning by acting on selective attention or alternatively on stimulus reinforcement[2]. The circuitry in the mammalian brain that regulates these behaviours is, however, so complex that it is difficult to investigate the facilitatory or depressing effects of a neuromodulator in terms of its influence on specific synaptic connections.

Arthropod preparations

In animals such as the crayfish, the lobster and various insects, the circuitry is much more accessible and the cellular mechanisms by which neuromodulators induce behavioural changes can be investigated directly. These animals display some sophisticated behaviours, the generation of which is reasonably well understood at the neuronal level.

The arthropod central nervous system (Fig. 1) is built on a clearly segmented plan and contains many neurons that can reliably be identified from one preparation to another. These are amenable to intracellular microelectrode recording and network analysis, and the interactions between modulatory substances and their target neurons and muscles can be studied on a cell-by-cell basis. In vertebrates the only comparable studies are of very simple behaviours, such as swimming in the lamprey[3].

Modulation of rapid-response circuits

In several invertebrate species the experimental activation of single interneurons can initiate complete behavioural acts. One of the best studied examples is the escape behaviour of the crayfish, which begins with a tail flip that propels the animals away from strong tactile stimulation of the tail fan at the end of the abdomen[4,5]. The inputs from the sensory receptors on the tail fan connect either directly or through a group of sensory interneurons with the lateral giant fibres that mediate the tail-flip response (Fig. 2*a*). When the tail fan is touched, the inputs evoke action potentials in the lateral giant fibres that in turn excite the motor neurons that drive the flexor muscles of the abdomen. The resulting tail flip pitches the animal away from the source of stimulation[4].

The threshold for triggering the response varies with the behavioural context: it becomes more difficult to evoke a tail flip when a crayfish is restrained or when it is feeding, and the threshold for triggering action potentials in the lateral giant neurons goes up under these conditions. In the sensory pathways, sensitivity can be altered by repeated tactile stimulation which leads to a gradual decrease in the probability that the reflex will occur. This so-called habituation results from depression of the synaptic transmission between the tactile input neurons and sensory interneurons[4]. Conversely, the probability of eliciting a tail flip can be enhanced or sensitized, if the animal is subjected to strong electric shocks, because the firing threshold in the largest sensory interneuron decreases[6].

Both habituation and sensitization can be mimicked by applying biogenic amines to the nervous system: 5-HT reproduces the depressive effects of habituation, whereas octopamine mimics the sensitization produced by a traumatic stimulus. Neither amine elicits or affects the performance of the escape behaviours but, rather, they modulate synaptic transmission in the sensory circuits. The excitatory postsynaptic potentials in the lateral giants produced by stimulating sensory neurons are reduced when 5-HT is perfused through the arterial system and enhanced by octopamine perfusion[7] (Fig. 2*a*); destroying cells that are immunoreactive for 5-HT with a specific neurotoxin also removes the inhibitory effect of 5-HT on transmission at the synapses[8]. From such results, it can be assumed that 5-HT and octopamine have modulatory roles in the escape response.

Once it is initiated by the firing of the giant fibres, the escape response goes automatically to completion, so it seems reasonable that only the synapses in the input circuits should be modifiable. In other behaviours, such as aggressive and submissive postures in the lobster, several levels both centrally and peripherally are modulated simultaneously[9,10]. The injection of

5-HT into freely moving lobsters evokes a sustained flexion of limbs and abdomen resulting in an aggressive-looking stance, whereas injection of octopamine leads to sustained extension producing a submissive-looking posture[9,10]. The posture evoked by 5-HT results from the contraction of the postural flexor muscles, whereas octopamine produces contraction of the antagonistic extensors. Application of both amines at the peripheral synapses between motor neurons and muscles, however, induces a more vigorous response in both flexors and extensors, so the antagonistic actions of the two amines must result from modulation of circuits in the CNS (Fig. 2*b*). Electrophysiological recordings show that the spike frequency in flexor motor neurons is increased by 5-HT and decreased by octopamine, and vice versa in the extensor motor neurons. A corresponding antagonism is also found in the excitatory and inhibitory motor neurons that innervate each muscle (unlike vertebrates, arthropods have direct inhibitory innervation of their muscles).

Immunohistochemistry shows over 100 cells reacting with antibodies raised against 5-HT, widely distributed throughout the nervous system; experimental attention has focussed on two pairs of these neurons that have cell bodies in the first abdominal ganglion and fifth thoracic ganglion (Fig. 2*c*). Their morphology[11] provides some insight into how the opposing motor patterns could be coordinated by actions at both central and peripheral sites: each has two separate sets of endings, one in the peripheral neurosecretory tissue, where chemicals released can enter the blood, and the other in the neuropiles of the CNS, where integration of information for the activation of the flexor motor programme takes place. The specificity for the action of the amine must be in the CNS, whereas the periphery is simply primed to be more responsive to the central command[9-11].

Modulation of transmission at the neuromuscular junction may be quite widespread. Cockroaches, locusts and crayfish all have some motor neurons that release the pentapeptide proctolin[12-14], as well as a classical excitatory transmitter, which is probably glutamate[15-17]. Proctolin does not depolarize the muscle membrane if applied alone; even though its detailed action is different in each animal, its general affects are to enhance contractions evoked by application of glutamate, or to cause sustained tension instead of a rapid twitch.

Selection of activity patterns

Many rhythmic behaviours result from patterns of activity in central pattern generators (CPGs) which consist of neuronal circuits capable of producing motor output patterns in the absence of sensory feedback. Sensory inputs are nevertheless essential for the activation and shaping of a precisely coordinated motor output. In the lobster, food is ground by three teeth in the anterior part of the stomach, known as the gastric mill, and is then pumped and filtered by the rhythmic constrictions and dilations of the posterior or pyloric end of the stomach. The muscles involved in the movements of the stomach are controlled by a CPG of about 30 neurons located in a small nerve centre termed the stomatogastric ganglion, together with some neurons in the commissural ganglia[18,19]. The rhythm-generating circuits in the stomatogastric ganglion are capable of producing many different output patterns in response to different transmitters in the input fibres[19,20] (Fig. 3), allowing a cellular analysis of the interactions between neuromodulators and their target neurons. Using an endoscope and video recordings, rhythmic patterns in the outputs of the isolated stomatogastric ganglion have been related to the movements of the gastric teeth in the intact animals[21]. Injections of proctolin into the blood of an intact animal alters the integration in the CPG circuit in such a way that several modes of chewing and grinding movements are produced in a dose-dependent manner. Neuromodulators such as proctolin may not only influence which output is produced but also modulate the strength of the interaction between the gastric and pyloric burst-generating

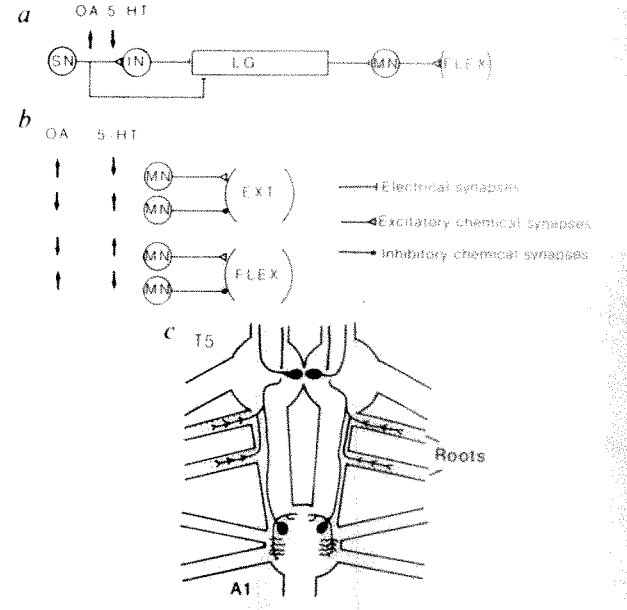


Fig. 2 Neuromodulation of rapid response circuits in crustaceans. *a*, In the crayfish tail-flip circuit, transmission at the synapses between the sensory neurons on the tail fan and the sensory interneurons is enhanced by octopamine and depressed by 5-HT and thus alters the threshold of the tail-flip response to tactile stimulation of the tail fan. *b*, Modulation of aggressive and submissive postures in the lobster by octopamine and 5-HT. The two amines have opposing effects on the excitation of the motor neurons of the flexon and extensor muscles. *c*, Schematic drawing of 5-HT immunoreactive cells in the lobster, with somata in the first abdominal ganglion (A1) or the fifth thoracic ganglion (T5). The cells innervate two separate release sites (stippled), a peripheral neurosecretory site in the nerve roots and central sites in the neuropile of the ganglion. SN, sensory neurons; IN, sensory interneurons; LG, lateral giant command fibre; MN, Motomeurons; Ext, Extensor muscles; Flex, fast flexor muscles; Drawings modified after refs 4, 6, 9, 10, 11.

circuits in the stomatogastric ganglion, thus promoting the integrated activity of the stomach when dealing with foods of different qualities.

Orchestration of behavioural units

Fully coordinated sequences of behaviour in several insects can be released by the injection of transmitters or their analogues either into the haemolymph or directly into the nervous system. In general, octopamine has a releasing or enhancing effect that is opposed by 5-HT.

Octopamine not only promotes the expression of a behaviour in insects but also helps to integrate performance at several levels of organization. In restrained locusts, for example, flight motor activity can be initiated by the iontophoretic application of octopamine into the neuropile of the metathoracic ganglion[22,23]. Octopamine also influences the kinetics of contraction in flight muscles[24,25], resulting in an energy saving increase in power output. During long flight sequences, octopamine induces the release of the adipokinetic hormones from stores in the corpora cardiaca, resulting in mobilization of lipid (see Fig. 1)[26]. Thus, this biogenic amine not only induces flight motor activity in the CNS but also mobilizes the metabolic fuel required for flight.

The main candidates for neurons that release octopamine in the ventral nerve cord of locusts are a group of approximately 90 dorsal unpaired median (DUM) neurons (Fig. 1). Most of these are local interneurons but a few of the larger cells have axons that bifurcate to innervate the musculature on both sides of the body[27-30]. Sombati and Hoyle[22] proposed that the release of octopamine from DUM neurons is causally related to the initiation and modulation of behaviour, because iontophoretical

administration in the appropriate ganglia elicits flight or rhythmic stepping movements of the legs, or suppresses the abdominal digging rhythm used by females during egg laying. As electrical stimulation of single DUM cells does not elicit any behaviour[22,31], the probability that a behaviour will occur may depend on the pattern of activity over the whole population of DUM cells. The resulting pattern of release in the neuropile could thus determine "which parts of which circuits are most likely to be thrown into (or out of) action"—the so-called orchestration hypothesis[22].

Indeed, application of octopamine and its receptor agonists, such as the formamidines, have strong behavioural effects; nocturnal moths become hyperactive during daytime[32], the male cabbage looper moth becomes hypersensitive to the olfactory signal that induces sex-pheremone mediated flight, and engorged ticks detach from their hosts[34]. In the fly and the bee, octopamine or its agonists enhances the reflex response to stimulation of sugar receptors on the feet or antennae with sucrose and causes the animals to feed twice as much as the controls (refs 35, 36 and R.M. and P. Schulz, unpublished results). Because formamidines disturb behavioural co-ordination[37-38], they may prove to be potent insecticides.

The antagonistic effects of octopamine and 5-HT have been demonstrated by injecting the amines into the haemolymph in several preparations. Octopamine and 5-HT induce opposite postures in males of the cabbage looper moth when they attempt to take off to fly in response to a sex pheromone[33]. In honeybees the two biogenic amines may act antagonistically in the visual system. In response to moved striped patterns, bees make direction-specific adjustments of antennal position, a reflex which is enhanced by application of octopamine and reduced by 5-HT in a dose-dependent fashion[39]. In flight behaviour, different amines may regulate different aspects of the control system: in the hawkmoth *Manduca*, octopamine evokes flight sequences, presumably by enhancing the efficacy of sensory transmission, whereas dopamine seems to have a direct excitatory effect on the interneurons of the flight motor and 5-HT surpresses flight motor output[40].

Hormonal orchestration of development

The natural release of hormones during the life cycle of an animal provides the most striking examples of the orchestration of behaviour by circulating chemicals over a time span of hours to days. In insects, hormone levels are known to affect reproductive behaviours, such as courtship in crickets[41] or the division of labour in the honeybee hive[42].

A cellular approach to hormone-mediated behavioural changes during development has been possible in the moth *Manduca*, which undergoes a complete metamorphosis from the larval or caterpillar stage, through the pupa to the adult[43,44]. The transition through the various stages is regulated by the relative levels of ecdysteroid hormones and juvenile hormones in the haemolymph. A third hormone, eclosion hormone, regulates the final shedding of the pupal cuticle and emergence of the adult, a process termed eclosion. This consists of two phases, the splitting of the cuticle over the thorax by rhythmic movements of the wing bases; and peristaltic waves of the abdomen, which propel the shed cuticle posteriorly. Both phases are centrally programmed, because the appropriate motor patterns can be triggered when the isolated nerve cord is exposed to eclosion hormone.

The nervous system is sensitive to eclosion hormone only shortly before the normal time of hatching. A circadian clock in the brain gates the release of eclosion hormone from neurosecretory centres located in the brain[43,44]. Eclosion hormone is released as a pulse 2.5 hours before the adult emerges and during this period neurons in the nerve cord become sensitive to the hormone as they start to express two proteins that may be required for the transduction of its signal[45]. The control of the expression of these protein requires the presence of

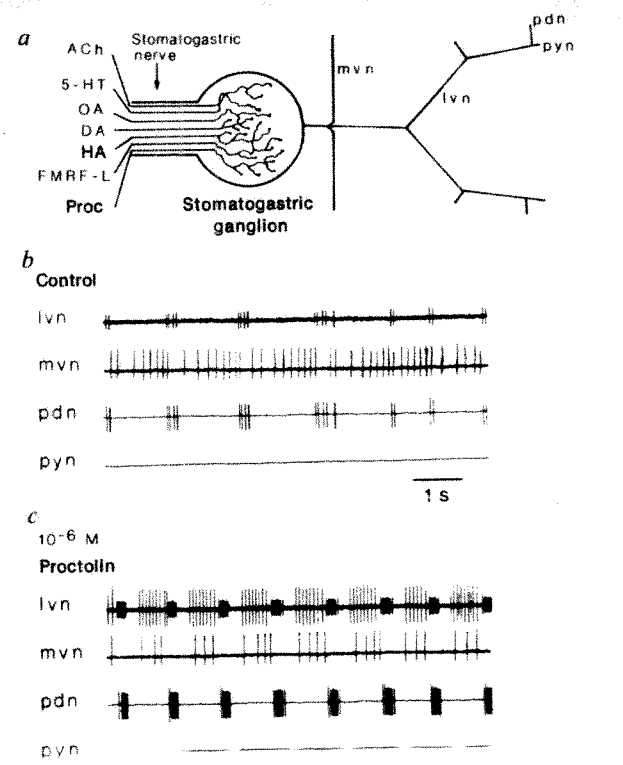


Fig. 3 Neuromodulation of rhythmic motor output of the crustacean stomatogastric nervous system, which control the coordination of the muscles of the stomach. *a*, The stomatogastric nerve carries inputs to the stomatogastric ganglion from other ganglia that use a variety of transmitters. Each transmitter elicits a different motor pattern. Approximately half the cells in the stomatogastric ganglion contribute to the central pattern generator (CPG) for the rhythm of the gastric mill and about the same to that for the pyloric rhythm, both of which can be activated in the isolated ganglion. There is only one interneuron in each of the gastric and pyloric CPGs; the rest of the neurons of the CPGs are motor neurons. The pyloric pattern is an emergent property of the synaptic connections and membrane properties in the network of interneuron and motoneurons and the pacemaker activity of the anterior burster interneuron[18,19]. *b*, An example of pyloric motor patterns generated by the isolated ganglion, recorded extracellularly from the median ventricular nerve (mvn), lateral ventricular nerve (lvn), pyloric dilator (pdn), and pyloric nerve (pn) shows the modulation of the pyloric rhythm (*c*) when proctolin is added to the bath. It produces an increase in cycle frequency and a change in phase relations in the firing of pyloric motor neurons, which would result in a different pattern of contraction in the pyloric muscles (20). Comparing the effect of proctolin on synaptically isolated neurons with those in the intact network shows that the modulatory effect cannot solely be attributed to direct effects on pyloric neurons (20). Rather, the response of the circuit is shaped by connectivity among neurons that are directly affected and those that are unmodulated ACh: acetylcholine; 5-HT: serotonin; OA: octopamine; DA: dopamine; HA: histamine; FRMF-L: FRMF-like peptide; Proc: proctolin. Modified after refs 19, 20.

ecdysteroid hormone, but also its subsequent removal[46]. Timing is also essential for the sequential activation of the two phases of eclosion behaviour, brought about by a different latency of the action of eclosion hormone on the motor circuits controlling the two phases.

If the pupal cuticle is peeled off before the animal enters the eclosion period, adult behaviours such as walking and the righting reflexes are absent. These appear with a delay of several hours if the eclosion hormone is injected, transforming the prematurely born, uncoordinated animal into an adult with a normal behavioural repertoire[43]. The changing levels of hormones thus orchestrate profound cellular changes during post-embryonic development that are manifested in stage-specific behaviours. And not only do they affect the responses of neurons

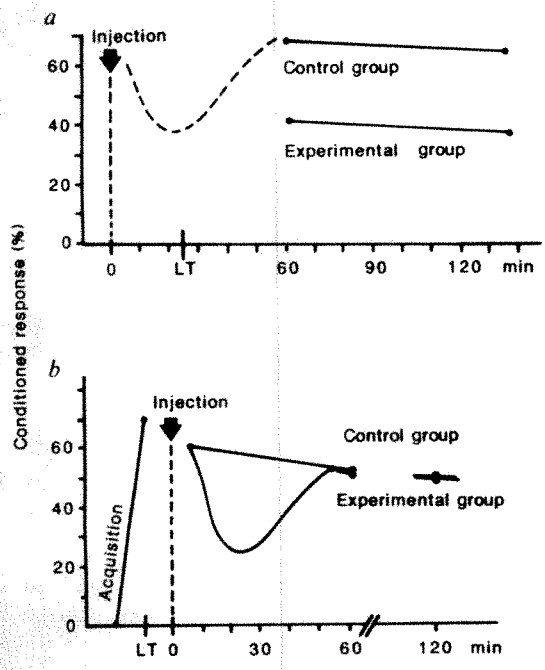


Fig. 4 The experimental procedures used to study the effect of drugs injected into the brain of the honeybee on learning, memory and retrieval from memory. The basic learning procedure is to test a group of bees for their spontaneous responses to an odour and then to condition the non-responding animals once to this odour by pairing the odour with sucrose solution (the learning trial, LT). After conditioning, about 70% of the animals extend the proboscis to the presentation of the odour alone. *a*, Storage test: to see whether a drug interferes with the formation of memory, it is injected before the learning trial. After the drug or a saline control is injected, the animals are conditioned by one trial (LT) at the time when the action of the drug is optimal, as determined in the recall test (see below; dotted curved line indicates that this is a background effect not particularly tested in this experiment). The repeated presentations of odour alone follow when the immediate effect of the drug has vanished. The example given is for an injection of 5-HT (10^{-8} M, 5 nl) into each α lobe of the mushroom bodies (see Fig. 5): the experimental animal shows a persistent reduction in the conditioned response. This proves that 5-HT injected into the α lobe interferes disruptively with the formation of a memory trace. Other drugs like octopamine or noradrenaline may persistently improve the memory when injected before the conditioning trial (see Fig. 5, unpublished results reproduced by kind permission of M. Sugawa). *b*, Retrieval test: the drug is injected into the brain after the learning trial and the animals are tested by repeatedly presenting the odour stimulus alone. The control group (same amount of saline injected) shows a small decline in response, the extinction effect, which is due to repeated testing. The experimental group transiently responds less but the effect of the drug disappears at longer intervals. The example given here is for dopamine (10^{-6} M, 100 nl injected into the protocerebrum), which inhibits conditioned responding maximally at 20–30 min after injection. The animals can be successfully conditioned at a time when the action of dopamine is optimal, showing that the dopamine injection does not interfere with the sensory or motor components or those underlying memory formation[61].

to hormones: they also influence remodelling of neuronal circuits by incorporation of new neurons, reorganization of neuronal arborizations and cell death[47].

Behavioural plasticity and learning

As neuromodulators confer flexibility on neuronal circuits and trigger intracellular biochemical events that outlast the signal, they have been implicated in learning, memory and the performance of learned tasks in vertebrates[48–50], molluscs[51,52] and fruit flies[38,53,54]. There is some debate, however, about the site of modulatory action in associative learning.

Classical conditioning is a type of associative learning in which an animal establishes a connection between a neutral conditioned stimulus (CS), for example, a sound or coloured light and a reinforcing unconditioned stimulus (US) such as food. The repeated temporal pairing of the two stimuli during training elicits a response to presentation of the initially neutral stimulus that is normally caused only by the US. The association of events during classical conditioning requires a locus in the nervous system where the US and CS can converge. An analysis of classical conditioning at the cellular level in the mollusc *Aplysia* has pinpointed an allosteric regulation of an enzyme, membrane-bound adenylyl cyclase, as the most likely point of convergence[51]. It is stimulated by the release of a neuromodulator, 5-HT, in the US pathway and its activity is amplified by activity in the CS pathway, which increases intracellular calcium levels.

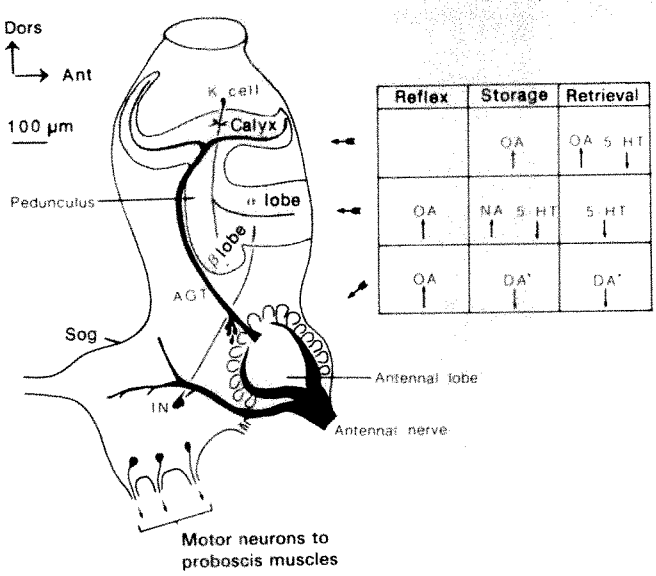
Some studies in the vertebrate literature, however, consider the US and CS pathways separately from the modulatory functions of biogenic amines, which are assumed to regulate affective states and thus are seen as a third and independent component in the neural substrate of learning[55,56]. Amines are thought to cause an arousal state in the memory-forming part of the nervous system, so directing 'attention' to the conjugation of stimuli.

The honeybee provides an excellent preparation for addressing this question[57–62]. Under restrained conditions in the laboratory a memory lasting for days is produced after a single trial in which the CS is an odour, paired with a sucrose reward as the US[63]. The animal extends its tongue (proboscis), its normal response to sucrose, as a conditioned response (CR) when an initially neutral odour stimulus is presented[57,64]. This learning process can be manipulated by global or local injections of small quantities of drugs (around 5 nl) directly into the brain. As learning occurs in a single trial that lasts only a few seconds, drugs can be used to separate memory formation from memory retrieval, particularly to examine the unresolved question of whether these are conceptually separate processes. By injecting drugs before the learning trial, storage can be disrupted; injection after the trial disrupts retrieval (Fig. 4).

As an example, consider the action of a global injection of dopamine (10^{-6} M). If it is injected before the learning trial and the animals are tested at a time when the action of dopamine should be optimal (storage test), learning is not impaired but injections after the learning trial (retrieval test, Fig. 4), induces a transient reduction in the conditioned response to the presentation of the CS alone[58,59,61]. Thus, dopamine interferes selectively with the retrieval process. Because performance in the storage test is unchanged, dopamine has no effect on olfactory coding, motor response or memory storage. In contrast, injections of amines such as 5-HT interfere with the storage process (Fig. 4*a*) leading to irreversible effects in the conditioned response[65]. As the motor performance during the period when the action of 5-HT is optimal is unaltered, 5-HT must interfere with the storage processes, not with the sensory or motor components of the task.

Local injections of biogenic amines into particular regions of the brain reveal a different pattern of action for each modulator that depends on the area injected and the behavioural components tested[62] (Fig. 5). For example, in the chemosensory processing area, the antennal lobes, octopamine seems to be involved in stimulus reinforcement. In the higher association areas, the mushroom bodies, octopamine enhances both memory storage and retrieval, whereas noradrenaline enhances only storage and only when injected into the output areas of the mushroom bodies. The facilitatory effects of both octopamine and noradrenaline are antagonized by 5-HT (see Fig. 5 legend for more details).

The most important outcome of these experiments is that learning and retrieval can be improved or depressed by different amines acting at different locations and at different times in the learning process. The experiments also support earlier conclusions[65,66] that the memory system in the bee brain involves



Reflex	Storage	Retrieval
	OA ↑	OA ↑ 5-HT ↓
OA ↑	NA ↑ 5-HT ↓	5-HT ↓
OA ↑	DA* ↑	DA* ↑

Fig. 5 The effect of local drug injections on the proboscis-extension reflex and on storage and recall of the conditioned response during olfactory learning in honeybees is summarized on a schematic side view of the main pathways in the brain and suboesophageal ganglion (Sog) mediating the proboscis extension reflex. Chemosensory afferents from the antenna terminate in the glomeruli of the antennal lobe. Relay neurons transmit olfactory information through the antenno-glomerular tract (AGT) into the calyx of the mushroom body. The mushroom body (stippled) contains approximately 170,000 intrinsic Kenyon cells (K cell), which project from the calyx to the two output regions, the α- and β-lobe[74]. Monoaminergic interneurons (IN) connect the suboesophageal ganglion with neuropiles around the α- and β-lobes. The suboesophageal ganglion contains the motor circuits and motor neurons responsible for the extension of the proboscis. The table summarizes the action of locally injected amines on the three components of behaviour—proboscis extension reflex, storage and recall. In the table, the arrows pointing upwards indicate facilitation of the reflex or conditioned response, those pointing downwards indicate a reduction. Bold arrows indicate the injection sites. Octopamine (OA) mimics the sensitizing action of the US (sucrose stimulus) and stimulates feeding behaviour when injected into the antennal lobe, suggesting octopamine has a role in reinforcement. If OA is injected into the pedunculus of the mushroom bodies close to the input synapses of the chemosensory relay neurons in the calyx, no changes are found in the reflex performance but both components of learning, memory formation and recall are enhanced. OA has no effect when injected into the output regions of the mushroom body, the α-lobes, whereas noradrenaline (NA) injections into this region enhance memory formation, particularly in animals with lower learning rates. 5-HT on the other hand antagonizes the facilitatory actions of OA and NA in the mushroom body but does not affect the non-associative modulation of the reflex. The substances (2.5 nl, 10^{-6}–10^{-8} M) were injected into either both antennal lobes, or both α-lobes or both pedunculi (Pd) of the mushroom bodies (the volume of the brains is ~1 μl). The drug effects were compared with control animals which were injected at the same sites with the same volumes of saline. Several hundred animals were tested[58,62]. Data for DA* from 60. Dors, Dorsal; Ant, anterior (orientations refer to the position of the brain in the head in life).

several regions, with non-associative modulation of the feeding reflex taking place in the antennal lobes, whereas the mushroom bodies, an area thought in other insect species to be involved in the coordination of behaviour patterns[67], are necessary for the long-term formation and read out of associative memory. Identified neurons immunoreactive for 5-HT that link these areas have been impaled with dye-filled electrodes[68], so it should be possible to analyse the learning capabilities of an insect brain at the cellular level.

The distinction between storage and retrieval mechanisms implies that the location of the memory trace is not the direct sensory pathway between the chemosensory afferents of the antennae and the motor circuit for the proboscis response in the suboesophageal ganglion (Fig. 5), because this pathway remains functional when the conditioned response is blocked pharmacologically. Anatomical studies reveal prominent fibre tracts that leave the direct sensory pathway and enter the mushroom bodies (Fig. 5). Storage of the memory trace in a pathway parallel to the main sensory–motor path contrasts the memory system of the bee with the model developed for associative and non-associative plasticity in molluscs, where activity-dependent modulation has been found at the synapses between sensory and motor neurons[51].

Chemical codes and behaviour

Both vertebrate and invertebrate nervous systems contain neurons with widespread branching patterns that seem suitable for coordinating neural activity in a large population of follower cells by a rather diffuse release of neuromodulatory substances. The investigation of some of these identified cells and their targets in arthropod nervous systems suggests that neuromodulators orchestrate behaviour by acting simultaneously at many synaptic levels in the nervous system to integrate neuronal activity, and also by more widespread regulation of metabolism. Sensory circuits may be sensitized or depressed, motor circuits can be biased towards favourable states, muscle tone is adjusted to the correct set point and the metabolism fuelling the muscle power is activated. This coordination of neuronal activity in different parts of the organism has also been observed in molluscs[69] and leeches[70], where identified amine-containing neurons integrate several appetitive and comsummatory components of feeding behaviour, such as food arousal, swimming toward food sources, bite frequency and meal size.

Experimental manipulation using neuromodulators or their analogues to mimic the natural release of neuromodulators can evoke coherent behaviour. The chemical code for regulating behaviour arises from the patterns of release of the various modulatory substances. These in turn are decoded by the distribution in the target tissue of receptors specific for each type of modulator. As the receptors are linked to the intracellular biochemical signalling pathways, the neuromodulators can modify the excitability of nerve cells on a longer time scale than that of conventional rapid synaptic transmission, leading to longer lasting functional changes in neural circuitry that serve processes such as arousal, selective attention, reinforcement and retention of memory.

Although the behavioural responses to neuromodulators are becoming increasingly better understood, the sensory pathways for activating neuromodulatory neurons are an enigma. A great deal of circuit chasing remains to be done to establish the connectivity between these neurons and external sense organs. Electrophysiological recordings from neuromodulatory neurons of motor circuits[11,22,31] has so far provided little evidence for a direct link to the mainstream of rapid sensory information processing. But it is conceivable that some neuromodulatory neurons may form, remote from sensory input, a central system for integrating the activity states of many neural circuits. Thus, a release of neuromodulators may mediate the translation of the activity pattern of many neurons into the activity in biochemical pathways in single target cells.

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ARTICLES

A unique geochemical record at the Permian/Triassic boundary

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A 330-metre core drilled through the marine Permian/Triassic boundary in the Carnic Alps of Austria allows closely correlated studies of geochemistry, petrography and palaeontology across the boundary. The isotope shifts and metal concentrations are extended, multiple and complex, and do not resemble those seen at the Cretaceous/Tertiary boundary. Both the carbon isotope shifts and the chemical events (including an iridium anomaly) may have causes related to a major regression of the sea.

THE Permian/Triassic (P/Tr) boundary is associated with the largest extinction event in Phanerozoic time[1]. It also witnessed important changes in the environment[2], notably indicated by a large drop in $\delta^{13}C$ of the surface ocean from the relatively enriched ^{13}C condition that characterized the late Palaeozoic[3,4]. A sudden decrease in $\delta^{13}C$ has also been observed in connection with other extinction events: the Precambrian/Cambrian[5], Ordovician/Silurian[6], Frasnian/Famennian[7], Devonian/Car-

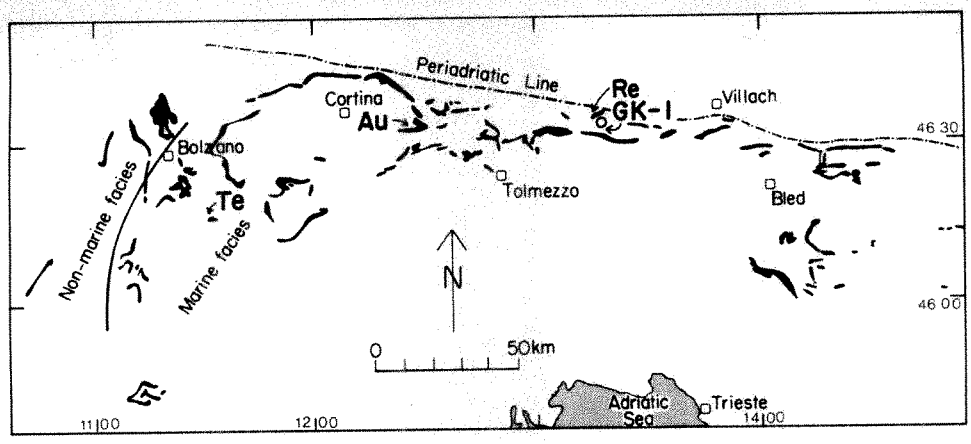


Fig. 1 Location of the Gartnerkofel-1 (GK-1) core hole in relation to the surface distribution of P/Tr rocks in the southern Alps[31]. Also shown are locations of the outcrop sections at the nearby Reppwand (Re), and at Tesero (Te) and Auronzo (Au), Italy[28]. The GK-1 locality is on a gently dipping marine shelf, 160 km east of the shoreline near Bolzano, Italy.

boniferous[8], Cenomanian/Turonian[9] and Cretaceous/Tertiary (K/T)[10]. At some of these boundaries, anomalous concentrations of metals, particularly iridium, have been observed. The high concentrations at the K/T boundary[11] and the more modest but distinct concentrations in the Late Eocene[12] have been widely ascribed to bolide impacts, whereas concentrations at some of the other extinction events[7,13-17], similar to those in the Late Eocene, may have been caused by terrestrial processes (biogenic, oceanic or volcanic).

Suggestions of iridium anomalies associated with the P/Tr boundary have been controversial. Notable iridium anomalies have been reported at exposures of the P/Tr boundary in China[16-19], but analyses of these same sections in other laboratories have failed to find iridium concentrations of more than 100 parts per 10^{12} (refs 20-23). Similarly low levels of iridium were found in P/Tr sections in Soviet Armenia[24] and in the Dolomite Alps[25,26].

Our objective in the current study has been to completely characterize the geochemistry and palaeoenvironment of a representative section of the P/Tr boundary, including carbon isotope and iridium characteristics as well as other measures, and to compare the conditions near the boundary with those in the contiguous sections of the Upper Permian and Lower Triassic.

The Gartnerkofel core

Previous studies of carbon isotope profiles of the P/Tr boundary have indicated that some of the most complete sections across the boundary were situated in the Southern Alps of Austria and Italy[27-29]. To obtain the best possible reference section free of any outcrop alteration, a continuous core was taken specifically for this purpose, on the north slope of the Gartnerkofel near Nassfeld in the Carnic Alps of Austria (Fig. 1). This locality was near the western end of the Tethys Sea, on a carbonate ramp gently inclined to the south-east and 160 km east of the shoreline exposed in the western Dolomite Alps[30,31]. Hole GK-1 started in the Muschelkalk Conglomerate (Upper Anisian), entered the Werfen Formation (Skythian) at 57 m, the Bellerophon Formation (Dzhufian–Dorashamian) at 231 m, and bottomed in the same formation at 331 m (Fig. 2). Oolites in the lowermost 6 m of the Werfen Formation correlate with the Tesero Horizon in the Italian Dolomites, where the P/Tr boundary, as defined by the last occurrence of Permian fusulinids[32-34], lies within this Tesero Horizon. In both GK-1 and the nearby outcrop on the Reppwand cliffs, conodonts were recovered from the lower 40 m of the Werfen Formation. The recognition of *Hindeodus parvus*, a conodont guide fossil for the Early Griesbachian[35,36], confirms a very thick and complete representation of lowermost Triassic sediments at this locality. The palaeomagnetic record in the core displays several polarity reversals, as seen in the Permo-Triassic Mixed (Illawarra) Superchron. The reversal nearest to the P/Tr boundary, from positive to negative polarity, lies within the Upper Permian (at 237.2 m); an analogous reversal in the P/Tr section at Shangsi, China, is close to the boundary within a sampling gap of 1.5 m (ref. 37).

The absolute time interval corresponding to the stratigraphic units outlined in Fig. 2 is difficult to assess. Units 1B to 3B—constituting a thickness of ~200 m—correspond to probably not more than one stage each of the Permian (Dorashamian) and Triassic (Griesbachian)[38], a time interval of ~5 Myr (ref. 39). This rate of ~40 m Myr^{-1} is equal to that calculated for the section at Shangsi[37], and is within the expected range for carbonate shelf sedimentation[40].

Closely coordinated sampling of the core allows comparison of a wide variety of data. About 300 billets were cut for thin-section preparation; the remainders of these billets were milled to powder and split for mineralogical, chemical and isotope analyses. Every one of the shaly or marly interbeds, about 50 in number and mostly a few millimetres thick, was similarly sampled and analysed. Samples were spaced in most of the core at a maximum distance of ~1 m, but were as close as 10 cm apart in the vicinity of the boundary. The complete scheme of sampling and analysis, and all the analytical results, are to be published elsewhere (W.T.H. *et al.*, to be submitted to *Jb. geol. Bund.*) Some salient features of the results are given here.

Carbon isotopes

The carbon isotope profile of Figs 2 and 3*a* suggests further subdivision of the section. Unit 1A, the middle part of the Bellerophon Formation (from the bottom of the core to 293.5 m), has ^{13}C-enriched values (mean $+3.0 \pm 0.3$‰). These values are within the range of those reported for Upper Permian rocks, both in other sections in the Alps[27,28] and throughout Tethys[29,41]. Unit 1B comprises the upper part of the Bellerophon Formation, where $\delta^{13}C$ values drop gradually from +2.8‰, to +1.5‰ at a depth of 231.3 m, at a rate of 0.02‰ m^{-1}. Comparison of this carbon isotope profile from GK-1 with others to the west at Tesero and Auronzo, Italy (Fig. 1)[28], indicates that this segment in GK-1 corresponds to the sharp drop in $\delta^{13}C$ that occurred within a few tens of centimetres in the Italy sections. In Unit 2 (the Tesero Horizon, which includes the P/Tr boundary) $\delta^{13}C$ continues to drop, reaching a value of 0.0‰ at 224.7 m. This segment has the same values of $\delta^{13}C$ and thickness as those of the Tesero Horizon at Tesero[28,42]. Unit 3A is an interval of low $\delta^{13}C$ in the lower part of the Mazzin Member of the Werfen Formation, extending to 175 m and including three minima, which will be discussed below. This multiple low was not found at any of the other P/Tr sections studied thus far. Units 3B and 4 comprise the upper part of the Werfen Formation, to 57 m, with a constant $\delta^{13}C$ of $+1.3 \pm 0.2$‰.

Oxygen isotopes

Values of $\delta^{18}O$ also show some consistent variations in certain intervals (Fig. 2). In Unit 1A the values of $\delta^{18}O$ show a bimodal distribution, centred at $\delta^{18}O = -0.5$ and -3.0‰, and the less

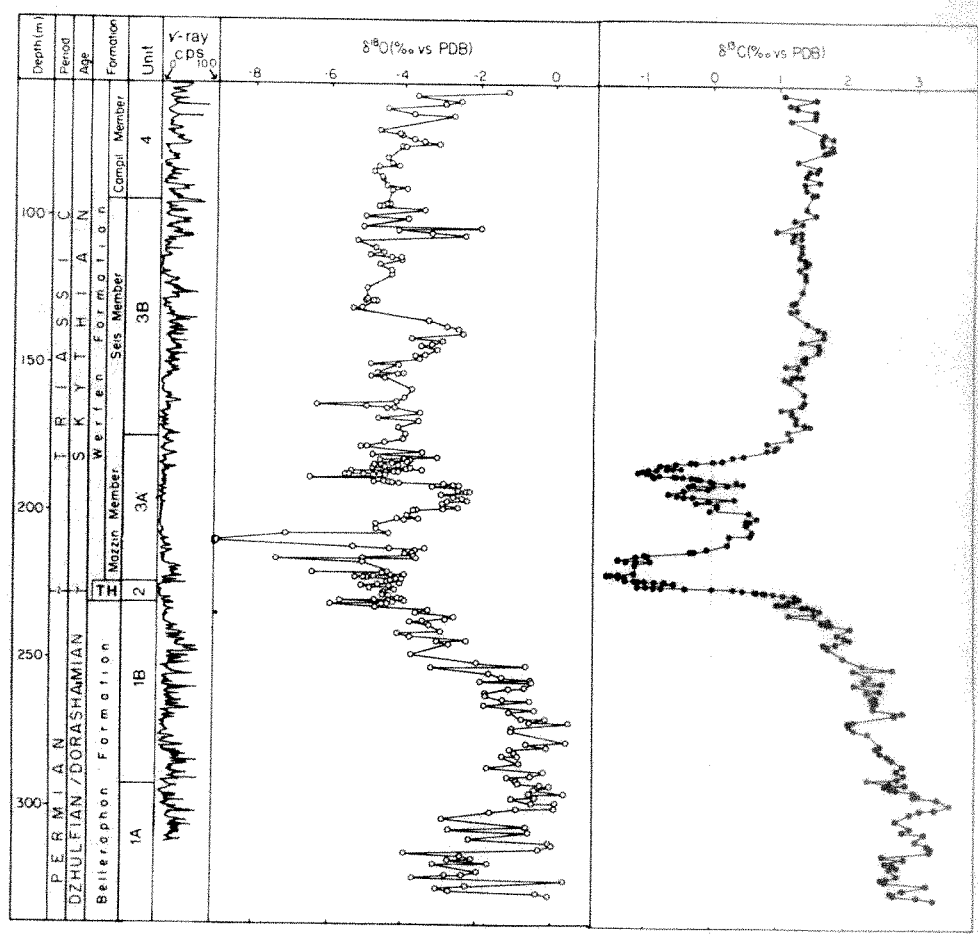


Fig. 2 Stratigraphy, geophysical log, and oxygen and carbon isotopes in GK-1. Four lithostratigraphic units are recognized, with subunits based on carbon isotope shifts. Virtually the entire section is fine-grained dolostone, with occasional millimetre-to centimetre interbeds of dolomitic marl or shale (natural γ-ray log >25 counts per second). Unit 1 is the middle and upper part of the Bellerophon Formation, a low-energy, homogeneous, bioturbated biomicrite of a shallow inner shelf, dominated by foraminifers. Unit 2 (231.0–224.5 m), the Tesero Horizon (TH), is transitional to a more shallow and restricted environment: oolites appear in the upper 4 m, with increasing tempestites, intraclasts, ostracodes and worm tubes. Unit 3 is the lower part of the Lower Triassic Werfen Formation, containing strongly bioturbated microsparite, with increasing coquina tempestites. In the interval 95–82 m the section becomes more red, less fossiliferous and richer in terrestrial clastics that characterize Unit 4. At the top of the section a hiatus of the higher parts of the Werfen Formation is followed at 75 m by the Anisian Muschelkalk Conglomerate (not shown). The clastic residues in Unit 1 are mainly illite/muscovite, whose measured crystallinity[67] attests to only very-low-grade diagenesis (<180 C). Upwards through Unit 3, detrital quartz and minor chlorite become dominant.

negative values continue in the lower part of Unit 1B. More negative $\delta^{18}O$ values of about −3.5‰ occur in the upper part of Unit 1B and persist consistently through Units 2 ($\delta^{18}O = -4.5 \pm 0.5$‰) and Units 3 and 4 ($\delta^{18}O = -4.2 \pm 0.8$‰), despite the substantial variations of $\delta^{13}C$ that characterize those intervals (Fig. 2). The consistent nature of the variations of $\delta^{18}O$ speaks for a mainly primary record of $\delta^{18}O$ in this core section. The depletion of $\delta^{18}O$ across the P/Tr boundary (Units 1 to 2 and 3) may be interpreted as indicative of a temperature rise of perhaps 5 °C.

Stable isotopes and chemistry across P/Tr

Detailed profiles of selected stable-isotope and chemical variations for the interval 235–175 m, which crosses the P/Tr boundary, are displayed in Fig. 3. This interval has complex variations of carbon isotopes and chemistry.

The smooth decrease of $\delta^{13}C$, which starts in Unit 1B, continues through Unit 2 at an accelerating rate, reaching 0.3‰ m^{-1}. This sharp drop in $\delta^{13}C$ signals the beginning of a zone of low $\delta^{13}C$ values, which includes three clear minima at 220 (−1.5‰), 193 (−0.6‰) and 186 m (−1.5‰), until a more stable regime with $\delta^{13}C \approx +1.3$‰ is re-established above 173 m (Fig. 2).

This ^{13}C-depleted zone is also characterized by chemical changes. The first and third carbon isotope minima have high concentrations of pyrite (>500 μmol g^{-1} S_2), as shown by dashed lines in Fig. 3*b*. Total Fe, shown as solid lines in Fig. 3*b*, is nearly matched (in μmol g^{-1}) by S_2 in these two pyritic intervals, underlain in both cases by oxidized intervals low in S_2, with most of the iron as goethite. Pyrite has wavy lamellar bedding consisting of globular masses 20–100 μm in diameter, similar to the early diagenetic 'framboidal' pyrite common in modern marine muds and in stratiform sulphide deposits such as the Kupferschiefer, Meggen and Rammelsberg[43,44]. Each of the stratigraphically distinct sulphide concentrations in Fig. 3*b* has a negative, uniform but distinct isotope ratio: for example, near 186 m, $\delta^{34}S = -19.3 \pm 0.2$‰ and at 221–222 m, $\delta^{34}S = -25.7 \pm 1.1$‰. These data indicate that the pyrite is syngenetic-diagenetic, and that each bed was deposited in its own distinct reducing environment. This conclusion is supported by rare-earth-element chemistry (Fig. 3*c*). Ratios of Ce^*/La^* have their highest values of ~1.0 in the intervals rich in pyrite, indicating anoxic conditions in the sedimentary regime[45,46]. Immediately below, in the interval 231–225 m, $Ce^*/La^* = 0.5 \pm 0.1$, as would be expected under the highly aereated conditions in which oolites are generated. The rise in Ce^*/La^* begins immediately above the oolite beds, 2 m below the beds in which considerable pyrite is preserved. Above the sulphide zone, Ce^*/La^* ratios (in shaly samples) decrease to intermediate values of ~0.7.

The iridium data also show two peaks (normalized to Al in Fig. 3*d*) that are substantially above background. The first (165 parts per 10^{12} Ir ($Ir/Al = 4.15 \times 10^{-9}$)), at the top of Unit 2 (224.5 m), occurs while $\delta^{13}C$ is still declining sharply towards its first minimum and the local conditions are just changing from oxic to anoxic. The second Ir peak (230 parts per 10^{12} ($Ir/Al = 6.80 \times 10^{-9}$)) is near the top of Unit 3A (185.6 m), centred on the third minimum of $\delta^{13}C$ and on a peak of S_2. The highest iridium concentrations are clearly above the relatively low background of iridium (10–30 ± 5 parts per 10^{12} in shaly samples) that apparently characterizes this geological period; they persist whether plotted as Ir/Al (Fig. 3*d*), Ir/Fe or just Ir. They are at least an order of magnitude lower than the iridium spike at the K/T boundary, but are similar to iridium concentrations described near other boundaries[7,13–15]. A log plot of Co against Ir (Fig. 4) shows that Permian/Triassic samples (including both our data from Austria and three analyses from China[21]) have lower Ir than (1) mean CI chondrite[47], (2)

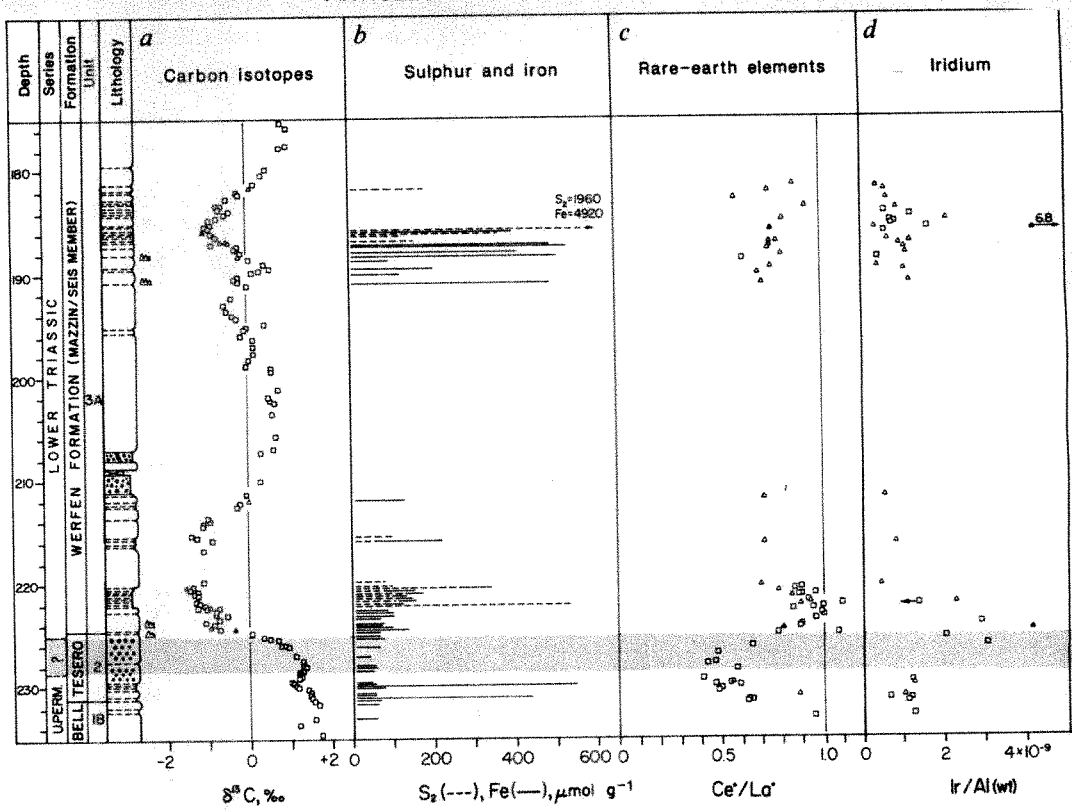


Fig. 3 Details of stratigraphy and geochemistry in GK-1, from 235 to 175 m, across the P/Tr boundary (shaded). Levels with *H. parvus* indicated alongside lithology. Squares represent dolostone, triangles represent shale or marl, and samples of the two iridium spikes are shown as solid symbols. *a*, Carbon isotope profile; note the steep but smooth decline of $\delta^{13}C$ across the P/Tr boundary, and the subsequent three minima in the lowermost Triassic; shale and carbonate samples are consonant. *b*, Total sulphur (nearly all is sulphide; analyses by combustion) and total iron (by instrumental neutron activation analysis (INAA)), expressed in $\mu mol\ g^{-1}$ of S_2 and Fe, respectively. In all cases the Fe is sufficient to indicate that all S appears as pyrite, FeS_2, which displays two peaks. *c*, Rare-earth elements (analysed by INAA), illustrated by Ce*/La*, each normalized to the North American shale composite[68]. Note the shift from low to high ratio (oxidizing to reducing conditions) at the upper boundary of the Tesero oolite. *d*, Iridium (analysed by RNAA), here normalized by its ratio to Al (analysed by INAA). The low background value of this ratio, at $\sim 1 \times 10^{-9}$, is maintained consistently through all the shaly layers and even above and below the section shown here. Two anomalies of many times the background level, at 4 and 7×10^{-9}, are seen at just above the P/Tr stratigraphic boundary as $\delta^{13}C$ passes through zero, and at about 40 m above the P/Tr boundary where $\delta^{13}C$ drops to its third minimum. Each of the two iridium anomalies rises sharply but drops off more slowly, over 1–2 m.

chondritic spherules from the deep sea[48], (3) chondritic spherules from the Tunguska event[49], and (4) peak spikes at the K/T boundary[50–51]. As indicated in Fig. 4, the ratios of Co/Ir in both background and in the nominal peaks of the GK-1 profile are more similar to those found in deep-sea muds[52] and manganese nodules[53,54]. Plots of Ni against Ir and Cr against Ir are similar to plots of Co against Ir.

Three samples from the upper Ir high were also analysed by radiometric neutron activation analysis (RNAA) for Au, and gave Au/Ir = 16, 30 and 56 (weight ratio). These are closer to the ratios found in basalts or kimberlites (summarized by Paul *et al.*[55]) than those in chondrites[47], K/T boundary clays[50] or deep-sea manganese nodules[53].

The weakest carbon isotope minimum (at 193 m; Fig. 3) is in a carbonate interval with no concentration of clay or pyrite, and has not yet been analysed for trace elements by NAA.

Geochemical measures of detrital provenance are constant, with no trend over the whole length of the core: $Th/Al = 0.161 \pm 0.028 \times 10^{-3}$ ($n = 76$) and $Ga/Sc = 1.70 \pm 0.36$ ($n = 25$). The latter ratio[56], and minor olivine and augite in heavy-mineral concentrates in Unit 3, suggest some input of fresh basic volcanic material, such as may have been derived from the Permian/Skythian Haselgebirge of the Northern Calcareous Alps[57].

Ten analyses of strontium isotope ratios ($^{87}Sr/^{86}Sr$) in carbonate rocks between 238 and 185 m (Units 1B, 2 and 3A), corrected for small amounts of radiogenic rubidium, had a mean value of 0.70742 (with a range of 0.70702–0.70777), similar to latest Permian[58] and earliest Triassic[2] rocks of western USA, but with no local evidence of an upward trend.

Modelling chemical changes

Taking into consideration the evidence presented here and that in earlier studies[2,3,28,29], we suggest the following model for changes that occurred in the environment across the P/Tr boundary.

The end of the Permian was characterized by one of the strongest regressions of the sea in the entire Phanerozoic[2], which explains why part of the boundary beds are missing in most of the marine sections around the world. Moreover, the thickness of the section in GK-1 that yields *H. parvus* (Fig. 3*a*) suggests that the Nassfeld area has a rather complete representation of 'boundary beds'. Thus the chemistry of GK-1 provides a very detailed record which substantially constrains the geochemical model.

A major shift in the carbon cycle was initiated early in the Dorashamian stage, based on evidence in sections across Tethys[29]. Thus the drop in $\delta^{13}C$ that begins in GK-1 (at least 50 m below the P/Tr boundary) indicates a net oxidation of the world reservoirs of organic carbon, as sea level dropped, exposing paralic and shallow shelves to erosion. The $\delta^{13}C$ profile in GK-1 confirms an acceleration in the rate of organic carbon oxidation as the boundary is approached, as was seen previously at Tesero, Italy[28]. At both Tesero[42] and in GK-1 (Fig. 3) the occurrence of oolites suggests that the sea had withdrawn to nearly expose these parts of the Tethys shelf.

A sharp change to anoxic conditions is evident at the top of Unit 2 in the GK-1 core by the rise in Ce*/La* and then the appearance of pyrite and high S/C ratios. The inception of anoxia, as $\delta^{13}C$ was dropping through zero for the first time, was marked by anomalous concentrations of iridium and other metals, and the maximum of the anoxic zone corresponds to the initial minimum in the profile of $\delta^{13}C$. A similar sequence is repeated during a later minimum of $\delta^{13}C$ at the top of Unit 3A (185.6 m), which is accompanied by another high concentration of metals and sulphide.

A (single) minimum zone of $\delta^{13}C$ at the base of the Early

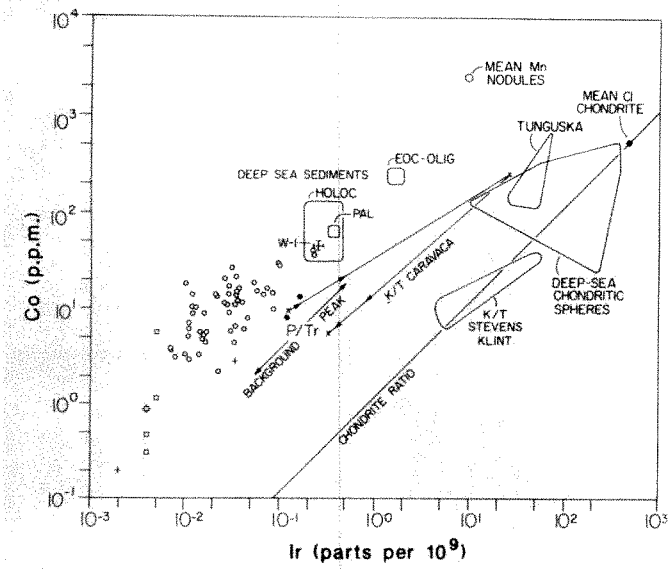


Fig. 4 Plot of cobalt against iridium on a log-log plot. Data from this study (from the Carnic Alps) are indicated as squares (dolostone) or circles (shale or marl); symbols for the two iridium spikes are solid. The data show a general positive correlation, but at a relatively lower Ir value than that for mean CI chondrite[47] or chondritic spherules[48,49]. Shown for comparison are two values for the P/Tr boundary beds in Changxing, China[21] (+), and two examples of the K/T peak (transition through the spike at Caravaca, Spain[50], marked ×, and the range of peak values at Stevens Klint, Denmark[51]). Also shown are approximate ranges of background for Palaeocene and Eocene-Oligocene (ref. 69 and F. T. Kyte and K. T. Wasson, personal communication) and Holocene (Ir: ref. 52; Co: ref. 70) deep-sea sediments, and mean values for manganese nodules (Ir: ref. 53; Co: ref. 54).

Triassic has been verified on a global scale[3,29]. The chemical changes that we see in GK-1—high iridium, other metals, sulphide and cerium—are certainly an important local signal, although they have not yet been found elsewhere. Whether they are local or worldwide, one must ask why these chemical changes have followed so closely upon the primary, worldwide, longer-term shifts of the carbon cycle. The explanation may be that both the carbon isotope shift and the chemical changes were caused by the same regression of sea level, which first exposed organic carbon to oxidation and finally brought about anoxic conditions to precipitate metals in at least this part of the Tethys Sea. This is consistent with increased erosion of upraised red beds[59] and volcanics[60], now exposed west of Bolzano (Fig. 1), bringing metals into solution which were finally reprecipitated on contacting the anoxic waters that had been generated in Tethys. Alternatively, the metals may have been dissolved from the red beds of the underlying Gröden Formation, and precipitated at the oxidation/reduction interface, as has been proposed for the metal-rich (including platinum-group elements) shales of the Kupferschiefer[61–63].

Accumulation of metals may have been aided by algae, whose presence is suggested by the lamellar textures seen in this interval. If detailed investigations of other sections show that anoxia and concentrations of metals are localized in this Bellerophon basin, one might then suggest that the cause is an estuarine-style topflow of the fresh waters from the lands to the west during the maximum regression of the Tethys Sea. Alternatively, on a larger scale it could represent a final phase of a deep-sea oxygen-minimum zone that was prevalent for much of the Palaeozoic[64,65], brought near the surface during this maximum regression. The prevailing incidence of fresh water eroding continental rocks during this period of regression is evidenced by the high values of $^{87}Sr/^{86}Sr$ compared with those of earlier Permian time[2], but we have not seen a short-term positive spike such as that seen at the K/T boundary[66].

Unlike other boundaries, for which a single rather sharp drop in $\delta^{13}C$ is followed by a relatively rapid recovery to normal $\delta^{13}C$, we found in GK-1 a thick section (Unit 3A, about 50 m) of low $\delta^{13}C$, including three separate minima, the two largest of which were characterized by anoxic conditions and enrichment of metals including iridium. Isotope profiles presented previously[28,29] have indicated that important worldwide changes in the carbon cycle had already begun in Late Permian time and continued smoothly across the stratigraphic P/Tr boundary to culminate in the Early Triassic. The detailed study of GK-1 confirms this extended interval of change, and presents a complex picture of oxidation, reduction and metal deposition constrained within the interval of carbon variations. The evidence presented here, from the most dramatic of all mass extinction events, provides a scenario that is completely different from the bolide impact model proposed for the K/T event, and which is less dependent on iridium anomalies. Certainly, a sharp event or events such as are seen at the K/T boundary are not in evidence here.

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GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in *Escherichia coli*

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Assembly of foreign prokaryotic ribulose bisphosphate carboxylases (Rubiscos) in Escherichia coli *requires both heat-shock proteins groEL and groES. GroEL is related to a chloroplast protein implicated in Rubisco assembly. Bacteria and chloroplasts therefore have a conserved mechanism that uses auxiliary proteins to assist in the assembly of Rubisco.*

THE subunits of some oligomeric proteins lack the inherent ability to correctly assemble into biologically functional molecules[1–4]. For the post-translational assembly of these polypeptides into oligomeric structures a ubiquitous class of conserved proteins, termed 'chaperonins', is thought to be involved[1]. This class of proteins includes the *E. coli groEL* gene product[1], as well as homologous proteins in other bacteria[5,6], chloroplasts[1], and mitochondria from plants, fungi and animals[7].

Both groEL and groES proteins are necessary for the morphogenesis of certain bacteriophages[8–12]. They are also involved in DNA replication in *E. coli*[13,14]. A molecular mechanism accounting for the involvement of the groE proteins in these processes is lacking however. The chloroplast homologue of groEL is called the Rubisco-binding protein because of its demonstrated association with newly synthesized chloroplast-encoded Rubisco large subunits[15,16], or with subunits imported into the organelle[17]. Both GroEL and Rubisco-binding protein have a similar structure and share extensive amino-acid sequence homology[1,18]. It has been suggested that in chloroplasts the Rubisco-binding protein is involved in assembly of hexadecameric (L_8S_8) Rubisco[19–21]. Here again, a detailed molecular mechanism is wanting.

Chloroplast Rubisco is comprised of eight large subunits and eight small subunits[22]. Rubiscos with a similar structure are present in most photosynthetic prokaryotes[23]. However, another form of Rubisco, a dimer (L_2) of large subunits, exists in the prokaryote *Rhodospirillum rubrum*[24]. The dimer from *R. rubrum* is similar in structure to a pair of large subunits of chloroplast Rubisco[25–27]. The structure of the hexadecameric chloroplast enzyme can be generated by tetramerization of *R. rubrum*-like dimers, to which the eight small subunits are polarily appended[26–28]. Both the prokaryotic L_2 and L_8S_8 enzymes can be synthesized and assembled in *E. coli*[29–32]. The demonstrated homology between the *E. coli* groEL protein and the chloroplast Rubisco-binding protein[1], led us to consider the possibility that the assembly of prokaryotic Rubisco in *E. coli* involves the bacterial groEL protein.

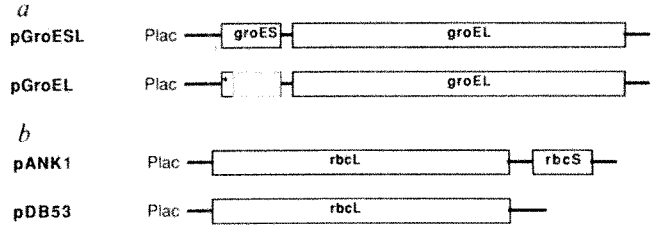


Fig. 1 Plasmid constructions. Two series of compatible plasmids were co-transformed in *E. coli* hosts. *a*, The first series (pGroESL and pGroEL) used plasmid pTG10, which has a p15A replicon, as a parent vector for the *groE* genes. pGroESL contains the *E. coli groES* and the *groEL* genes cloned in pTG10. pGroEL contains a frame-shifted (inactivated) *groES* gene followed by a wild-type *groEL* gene cloned in pTG10. *Translation frameshift. *b*, The second series of plasmids (pANK1 and pDB53) used pUC18 and pUC8 plasmids respectively, as parent vectors for the Rubisco genes. The pUC plasmids have a pMB1 replicon which is compatible with the p15A replicon. pANK1 (ref. 37) codes for both the Rubisco *rbcL* and *rbcS* genes from *A. nidulans*. cloned in pUC18 (ref. 38). pDB53 (ref. 31) codes for only the *A. nidulans rbcL* gene, cloned in pUC8 (ref. 39).
Methods. Plasmid pTG10 was derived from plasmid pACYC184 (ref. 40) which was cut with *Hin*dIII/*Bam*HI and repaired with Klenow. Into this repaired site was inserted a S1-nuclease-treated 433 base-pair *Hae*II fragment, containing the *lac* promoter and the multiple cloning site from plasmid pUC8. Plasmid pGroESL was made by cloning the *groE* operon, as a 2.3 kb *Eco*RI–*Hin*dIII fragment from plasmid pOF39 (ref. 13), into pTG10. Plasmid pGroEL was derived from a partial *Sac*II digestion of pGroESL, treated with T4 DNA polymerase. The resulting repair of the *Sac*II site located at codon 37 of *groES*[13] produced a frame-shift mutation.

Here, we show that assembly of prokaryotic dimeric (L_2), octomeric (L_8), and hexadecameric (L_8S_8) Rubiscos in *E. coli* requires both groES and groEL proteins. Although the groE proteins have been shown to mediate the assembly of L dimers from monomers, we cannot exclude subsequent involvement of these proteins in the formation of octomers and hexadecamers from these dimers.

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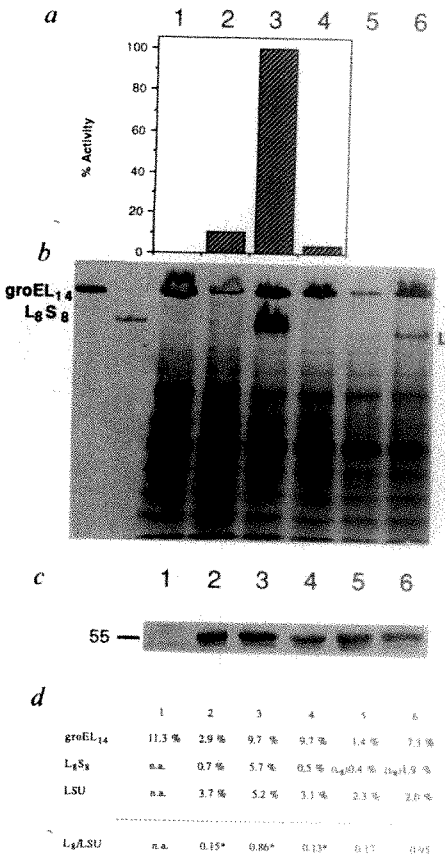


Fig. 2 *In vivo* assembly of Rubisco in *E. coli*. A *recA*56 isolate of strain MC1061 (ref. 41) was co-transformed with the following plasmids: (1) pUC18 and pGroESL; (2) pANK1 and pTG10; (3) pANK1 and pGroESL; (4) pANK1 and pGroEL; (5) pDB53 and pTG10; (6) pDB53 and pGroESL. *a*, Rubisco activity in the cell lysates. *b*, Non-denaturing 7% polyacrylamide gel electrophoresis of lysate proteins, strained with Coomassie Blue. Purified groEL[42] and spinach L_8S_8 Rubisco[43] are shown on the two left lanes. *c*, SDS-polyacrylamide gel electrophoresis of cell lysates, revealed by immunodetection with an antiserum raised against *A. nidulans* Rubisco large subunit. The large subunit appears as a band of relative molecular mass (M_r) 55,000. *d*, [^{35}S] counts incorporated in protein complexes from native and SDS-polyacrylamide gels as in *b* and *c*, respectively as a percentage of total TCA counts. n.a, not available; *, predicted L_8 values from L_8S_8 values; LSU, large subunit.

Methods. Cells grown on 2YT medium (16 g Bacto-Tryptone, 10 g Difco yeast extract, 5 g NaCl per litre) under selective conditions, were harvested at $A_{600} = 0.4$ and resuspended in 100 mM Bicine (*p*H 8.0), 10 mM $MgCl_2$, 30 mM $NaHCO_3$, 15% glycerol, 1 mM dithiothreitol, 15 mg ml^{-1} phenylmethyl-sulphonyl fluoride, 0.5 mg per ml lysozyme, 0.5 mg per ml deoxyribonuclease, 0.1 mg per ml ribonuclease. Samples were sonicated twice and centrifuged at 6,000 r.p.m. for 1 min at 4 °C. The protein concentration in the supernatant was measured using a Bio-Rad protein assay and adjusted to 5 mg protein per ml. The Rubisco activity was measured as the formation of acid-stable ^{14}C during a 20-min assay at 25 °C, using 100 μg of protein extract in 0.2 ml of 100 mM Bicine-NaOH (*p*H 8.0), 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.4 mM ribulose 1, 5-bisphosphate and 30 mM [^{14}C]$NaHCO_3$. (In the presence of both the pGroESL and pANK1 plasmids *E. coli* produced 0.26 nmol of active sites per mg protein as measured by [^{14}C]CABP binding[44]. 100% activity represents 8.0 nmol of CO_2 fixed per mg protein per min. This corresponds to a turnover number of about 0.5 s^{-1} and suggests that the enzyme may not have a full complement of small subunits). Non-specific $^{14}CO_2$ fixation was measured in the presence of 100 μM carboxy arabinitol bisphosphate, a specific inhibitor of Rubisco[33]. The results were corrected for this background fixation. In a separate experiment, cells were grown for 90 min in 2YT medium supplemented by 100 μCi ml^{-1} of [^{35}S] methionine, collected and lysed as above. Samples were analysed by non-denaturing PAGE on 7% gels and to SDS-PAGE on 15% gels. Bands containing the native groEL and Rubisco proteins were excised from the non-denaturing gel whereas the band containing the Rubisco large subunit was excised from the SDS gel. Counts of individual gel bands were normalized with values obtained for total cell lysates using precipitation in 10% cold trichloracetic acid.

GroE proteins stimulate Rubisco assembly

To demonstrate that groE proteins are involved in the assembly of Rubisco in *E. coli*, the level of cellular groE proteins was increased by cloning the *groE* genes on a multicopy plasmid. Two sets of compatible plasmids were constructed. The *E. coli groE* genes were cloned on a chloramphenicol resistant plasmid containing a p15A replicon (Fig. 1*a*), and the *Anacystis nidulans* Rubisco genes (*rbc*) were cloned on an ampicillin-resistant plasmid containing a pMB1 replicon (Fig. 1*b*). The presence of the plasmid encoding *groES* and *groEL* (pGroESL) in *E. coli* increased the amount of groEL protein from its usual level of 1–2% of cell protein, to about 10% (Fig. 2, *b*, *d*).

E. coli cells containing the plasmid encoding the *A. nidulans rbcL* and *rbcS* genes (pANK1) produced active Rubisco enzyme that accumulated to a level of 0.7% of cell protein during logarithmic growth (Fig. 2, sample 2). By increasing the cellular concentration of groE proteins with plasmid pGroESL, a substantial increase in Rubisco activity was observed (Fig. 2*a*, sample 3). This activity was inhibited (>98%) by the addition of carboxy-D-arabinitol 1,5-bisphosphate (CABP), a specific inhibitor of Rubisco[33]. An increased quantity of holoenzyme was also apparent on non-denaturing gels (Fig. 2*b*, sample 3). The identity of the gel band was confirmed by immunoblotting, and was found to correspond to 5.7% of the cell protein, as judged by the incorporation of [^{35}S] methionine (Fig. 2*d*, sample 3). Except for the groEL and Rubisco oligomers, the pattern of other proteins in Fig. 2*b* remained similar. Despite the 10-fold variation in active assembled holoenzyme, immunoblotting of SDS-polyacrylamide gels revealed that each of the cell lysates contained comparable quantities of Rubisco large subunit polypeptides (Fig. 2*c*, samples 2 and 3). The incorporation of [^{35}S]methionine into large subunits did not vary by more than a factor of ~2.5 (Fig. 2*d*). Therefore, the increased amount of assembled and active Rubisco arising from overproducing the groE proteins could not be attributed to increased expression of the Rubisco genes, or increased protein stability. We conclude that groE proteins are involved in the post-translational assembly of nascent Rubisco polypeptides into a functional holoenzyme.

On structural grounds, we believe that the final step in assembly of hexadecameric Rubisco is the addition of small subunits to a preformed L_8 octomer, and that small subunits are not involved in the assembly of this octomeric core. Evidence for this is provided by the observations that *A. nidulans* L_8 cores are produced in *E. coli* in the absence of small subunits[31], and that small subunits can be added to pre-formed cores to yield an active enzyme[34]. To examine the influence of groE proteins on the assembly of the L_8 cores alone, a plasmid (pDB53) was used which encodes the large subunit of *A. nidulans* Rubisco (Fig. 1*b*). When large subunit synthesis was directed by plasmid pDB53 in a strain with wild-type levels of groE proteins, a small amount of Rubisco L_8 cores were observed (Fig. 2*b*, sample 5). In a strain overproducing the groE proteins a significant increase in assembled L_8 cores was observed (Fig. 2*b*, sample 6). The limited activity of the L_8 cores in the absence of small subunits precluded measurements of enzymic activity in unfractionated lysates. However, titration with [^{14}C]CABP revealed that 0.14 nmol of sites were formed per mg of cell protein. Using the criteria described earlier, similar levels of Rubisco large subunit polypeptides were found between the various SDS-solubilized cell lysates (Fig. 2*c*, samples 5 and 6), which thus excluded the influence of the groE proteins on the expression or stability of the large subunit. Therefore, the groE proteins are involved in the conversion of nascent polypetides into L_8 cores.

Mutations in *groE* prevent Rubisco assembly

If groE proteins promote Rubisco assembly, then mutations in the *groE* locus should confirm the role of the groE proteins,

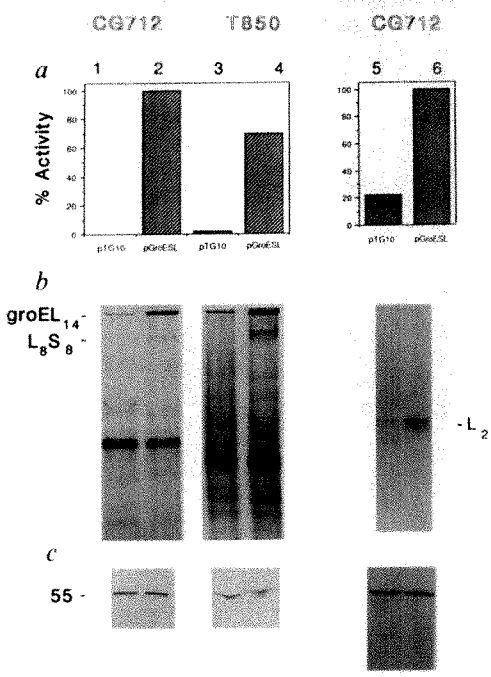


Fig. 3 Suppression by complementation of a deficiency in Rubisco assembly in strains of *E. coli* harbouring defects in the chromosomal *groE* operon. *E. coli groES*$^-$ mutant CG712 (ref. 13) or *groEL*$^-$ mutant T850 (ref. 45), harbouring *A. nidulans rbcL* and *rbcS* genes on plasmids pANK1 (samples 1–4) were co-transformed with the control pTG10 (samples 1 and 3) or with pGroESL (samples 2 and 4). The *groES*$^-$ mutant (CG712) was co-transformed with pRR2119 (fef. 29), which contains the *R. rubrum rbcL* gene, and with either the control plasmid pTG10 (sample 5) or with the pGroESL plasmid (sample 6). *a*, Rubisco activity in cell lysates, *A. nidulans*, samples 1–4; *R. rubrum*, samples 5, 6. 100% activity corresponds for *A. nidulans* Rubisco to 4 nmol CO_2 fixed per mg protein per min, and for *R. rubrum* Rubisco to 10 nmol CO_2 per mg per min. *b*, Non-denaturing PAGE (7% gels) of *E. coli* proteins, stained with Coomassie Blue (lanes 1–4) or revealed by immunodetection (lanes 5,6); *c*, SDS-PAGE (15% gels) of cell lysates, revealed by immunodetection with an antiserum raised against *A. nidulans* Rubisco large subunit M_r 55,000 (lanes 1–4) or against the *R. rubrum* enzyme (lanes 5, 6). The methods for preparing and analysing cell lysates are given in the Fig. 2 legend.

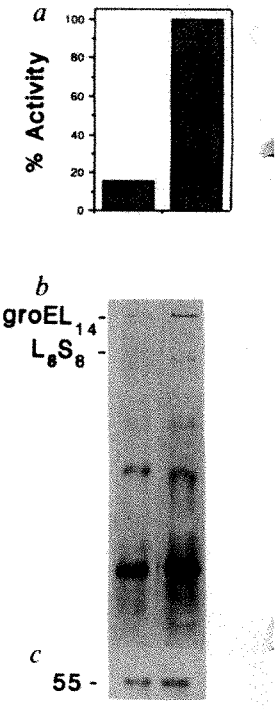


Fig. 4 Thermo-induction of Rubisco assembly. Wild-type *E. coli* cells (MC1061), harbouring plasmid pANK1, were grown either at 26 °C (left lane) or at 42 °C (right lane) to the cell density of A_{600} 0.4 from a common inoculum, initially grown at 26 °C, *a*, Rubisco activity in cell lysates (100% corresponds to 2.5 nmol of CO_2 fixed per mg protein per min) *b*, Non-denaturing PAGE (7% gel) of cell lysates stained with Coomassie Blue) *c*, SDS-PAGE of cell lysates in 1% SDS, 1% β-mercaptoethanol, revealed by immunodetection with an antiserum raised against *A. nidulans* Rubisco large subunit (M_r, 55,000). The methods for preparing and analysing the cell lysates are given in the Fig. 2 legend.

and perhaps identify the relative importance of groES and groEL in the process. Rubisco assembly was examined in *E. coli* strains with mutations in *groES* (strain CG712) or *groEL* (strain T850), or by using a groE overproducing plasmid (pGroEL) which had a frameshift mutation introduced into the *groES* gene (Fig. 1*a*).

When the *A. nidulans* Rubisco expression plasmid (pANK1) was transformed into CG712 (*groES*$^-$), neither Rubisco activity (Fig. 3*a*, sample 1) nor assembled holoenzyme (Fig. 3*b*, sample 1) could be detected. The compatible groES and groEL overproducing plasmid (pGroESL) was introduced into this strain to complement the mutation, which then allowed a significant production of active Rubisco holoenzyme from plasmid pANK1 (Fig. 3*a*, *b*, sample 2). A similar result was obtained using the *groEL*$^-$ strain T850. Virtually no Rubisco activity (Fig. 3*a*, sample 3) or assembled holoenzyme (Fig. 3*b*, sample 3) could be detected when T850 was transformed with the Rubisco expression plasmid (pANK1). When the groEL mutation of T850 was complemented by the presence of plasmid pGroESL however, both Rubisco activity (Fig. 3*a*, sample 4) and assembled holoenzyme (Fig. 3*b*, sample 4) were observed.

The influence of groES on Rubisco assembly was also demonstrated using the dual plasmid expression system. Figure 2 shows that an increased production of the groE proteins encoded by pGroESL led to improved assembly of hexadecameric (samples 2 and 3) and octomeric (samples 5 and 6) Rubisco. The *groES* gene of plasmid pGroESL was inactivated by introducing a translational frame-shift to produce plasmid pGroEL (Fig. 1). The presence of the *groES* frame-shift plasmid (pGroEL) and the Rubisco expression plasmid (pANK1) together in *E. coli* did not lead to improved assembly of Rubisco (Fig. 2, sample 4), thus emphasizing the involvement of groES in assembly of the hexadecameric enzyme. The *groES* frame-shift did not have a significant polar effect on expression of *groEL* because the plasmid-encoded *groEL* oligomer was present as an abundant protein (Fig. 2*b*, *d*, sample 4). Also, synthesis and stability of the Rubisco large subunit were unaffected (Fig. 2*c*, *d*, sample 4).

Recent structural studies of the L_8S_8 holoenzyme have revealed that the L_8 core is composed of four *R. rubrum*-like dimers arranged about a fourfold axis, the L_2 dimer being the basic structural and catalytically functional motif (see Fig. 5)[25–28]. This suggested that assembly of L dimers might be an essential intermediary stage in the formation of the L_8 core, and so the role of the groE proteins in this step was examined. The *groES*$^-$ *E. coli* mutant (CG712) was transformed with a plasmid (pRR2119) which codes for the *R. rubrum* Rubisco gene. Active, dimeric Rubisco could be detected in extracts from this strain (Fig. 3*a*, *b*, sample 5). However, the amount of active dimeric Rubisco was increased almost fivefold when the *groE* expression plasmid (pGroESL) was introduced into the *groES*$^-$ strain containing the *R. rubrum* Rubisco plasmid pRR2119 (Fig. 3*a*, *b*, sample 6). Again, the level of Rubisco subunits synthesized remained constant despite the different amounts of groE proteins produced (Fig. 3*c*, samples 5 and 6). The groE proteins are required for cell viability at 37 °C[1], and the mutant forms of groES and groEL present in CG712 and T850 would be expected to retain some residual or altered function. This may explain the presence of low levels of active Rubisco detected in *groE*$^-$ mutants (Fig. 3*a*, samples 3 and 5). The enhanced assembly of the L_2 dimer, observed by complementation and overexpression of *groES* and *groEL*, indicated that these proteins functioned at the very least at the stage of dimer formation.

Heat shock improves Rubisco assembly

In *E. coli* the expression of *groES* and *groEL* is stimulated following heat shock[35]. As assembly of Rubisco is influenced by the *groE* proteins, a heat shock to cells synthesizing Rubisco subunits might result in increased assembly of holoenzyme.

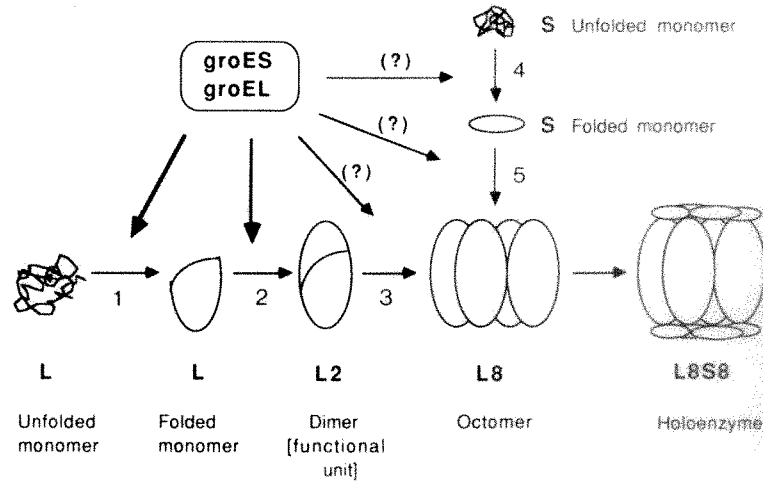


Fig. 5 Proposed assembly pathway of Rubisco hexadecamer. Recognizing that the L_2 dimer is the basic structural motif[25], a minimum of five steps must be invoked to convert nascent, unfolded large and small subunits into the hexadecameric L_8S_8 holoenzyme: (1) folding of large subunit monomer; (2) formation of the L_2 dimer; (3) tetramerization of L_2 dimers to give the L_8 core; (4) folding of small subunit monomer and (5) association of small subunits with the L_8 core. The groE proteins could potentially be involved at each step, although evidence[36] suggests that step 5 can occur spontaneously without their involvement. The results reported here show that, at the very least, the groE proteins are involved in dimer formation (that is, steps 1 and/or 2). However, the possibility that the groE proteins are additionally involved in steps 3 and 4 cannot be excluded.

A strain (MC1061) with wild-type chromosomal *groES* and *groEL* genes was transformed with the plasmid encoding *A. nidulans* Rubisco genes (pANK1). Cells were grown at 26 °C in liquid culture, and then inoculated into fresh media at either 26 °C or 42 °C. The heat shock increased the synthesis of groEL (Fig. 4*b*), and also resulted in a sixfold increase in Rubisco specific activity (Fig. 4*a*). Production of the L_8S_8 holoenzyme was also enhanced (Fig. 4*b*), but there were comparable amounts of large subunits produced at both temperatures (Fig. 4c). When the experiment was repeated with a *groEL*$^-$ strain (T850), only background levels of assembled enzyme were obtained (data not shown). The stimulation of Rubisco assembly by heat shock therefore required a functional groEL protein. The pleiotropic nature of the heat-shock response requires some caution in interpretation of these experiments. The results are consistent however with the *groE* overproduction experiments and use of mutants described earlier.

Discussion

The experiments described here demonstrate that the groES and groEL proteins are both obligate accessories for the assembly of prokaryotic Rubiscos in *E. coli*. The assembly of dimeric, octomeric and hexadecameric Rubiscos are all influenced by the groE heat-shock proteins. There are several potential sites of action for groE proteins in the assembly of Rubisco (Fig. 5). These sites of action might be non-exclusive and could be at the steps of polypeptide-chain folding to form L and S monomers, dimerization of L monomers, tetramerization of L dimers, or adjunction of small subunits to the L octomer. Experimentally, we have shown that the groE proteins are required for assembly of the *R. rubrum* L dimer. Dimerization of L could be the basic common step involved in the assembly of the L octomer and the L_8S_8 hexadecamer of *A. nidulans*. Addition of small subunits to the L_8 core does not seem to require groE proteins, as this step can be accomplished *in vitro* with no additional protein factors[36]. We cannot exclude a role for the groE proteins in the folding of the small subunits.

Our data shows for Rubisco, and implies for other proteins, that the primary structure of some oligomeric proteins is insufficient to specify their spontaneous assembly into a biologically active form *in vivo*. The dependence of a foreign oligomeric protein on host heat-shock proteins for correct assembly provides experimental evidence for the existence of a conserved mechanism capable of controlling the assembly of a variety of oligomeric proteins. This has also been inferred by structural comparisons of chaperonins[1].

The requirement for groES in Rubisco assembly in *E. coli* is of particular interest when considering the biogenesis of organelles in eukaryotes. A groEL homologue is present in chloroplasts and mitochondria[1,7], and has been implicated in the assembly of plant Rubisco[19–21]. The sequence and functional conservation of groEL in protein assembly leads us to predict that a protein with a function analogous to groES may be present in organelles to assist the post-translational assembly of some of their proteins.

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A second supernova inside Puppis A?

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Young supernova remnants provide rare glimpses of material from the cores of massive stars, leading to observational tests of stellar evolution and nucleosynthesis theories. Here we present narrow-band images of an unusual swirl structure located near the centre of the Puppis A supernova remnant, which reveal three overlapping filamentary systems of highly diverse composition. One is dominated by emission lines of nitrogen, one by oxygen and one by sulphur. Spectra indicate that these small rings have high velocities and a corresponding kinematic age of less than 800 yr. Heavy-element abundances are extremely high and different in each system, some reminiscent of knots in Cas A, and others of circumstellar matter observed surrounding supernova 1987A. The youth and unusual chemistry lead us to suggest that a second supernova has exploded within the shell of Puppis A, giving rise to the swirl structure. A similar suggestion was made by Elliot *et al.*[1], based only on the region's appearance in Hα.

The irregular shell structure of Puppis A, about 50 arcmin in diameter, is apparent at both radio[2] and X-ray[3] wavelengths. Its bright optical filaments lie predominantly near the shell's rim to the north-west and to the east (Fig. 1). Spectra of these bright filaments show emission lines typical of supernova remnants (SNRs), although some are notable for very strong nitrogen lines[4-6]. Measured radial velocities[7,8] for these filaments have without exception been modest, $v_r < 300$ km s^{-1}. In striking contrast are a recently discovered[9] set of faint filaments which have strong oxygen lines, almost no Balmer emission, and radial velocities as high as 1,500 km s^{-1}. Proper motions for the latter filaments are consistent with undecelerated expansion from a common origin 3,700 years ago[10]. The explosion of a massive star ~4,000 years ago is consistent with all previous data from Puppis A at X-ray, optical and radio wavelengths.

Our new observations come from a complex region[1] which comprises filamentary systems of three different types, two of which have not been previously observed in Puppis A. This structure is centred near $\alpha = 8^h20^m56^s$, $\delta = -42°50'$ (1950), 3 arcmin south-east of the centre from which the fast oxygen-rich filaments are expanding[10], and appears as a swirl of emission ~4 arcmin in diameter—less than one tenth of that of Puppis A—on the deep Hα + [N II] plate shown in Fig. 1. No excess emission of the swirl region over its surroundings is seen in radio[2] or X-ray[3] maps of Puppis A.

We used the prime-focus CCD camera with an RCA chip at the CTIO 4-m telescope on 17 February 1988 to obtain narrow-band images of the swirl region at wavelengths of three prominent emission lines plus a continuum image (see Table 1 for details). The four images are shown in Fig. 2. Each of the emission-line images shows a filamentary structure typical of SNRs, yet the images in the different bands are so dissimilar that it would be difficult to recognize them as the same object, were it not for the star field.

Two nights later we used the Ritchey-Chretien spectrograph and 2D-Frutti detector on the CTIO 4-m telescope to obtain a

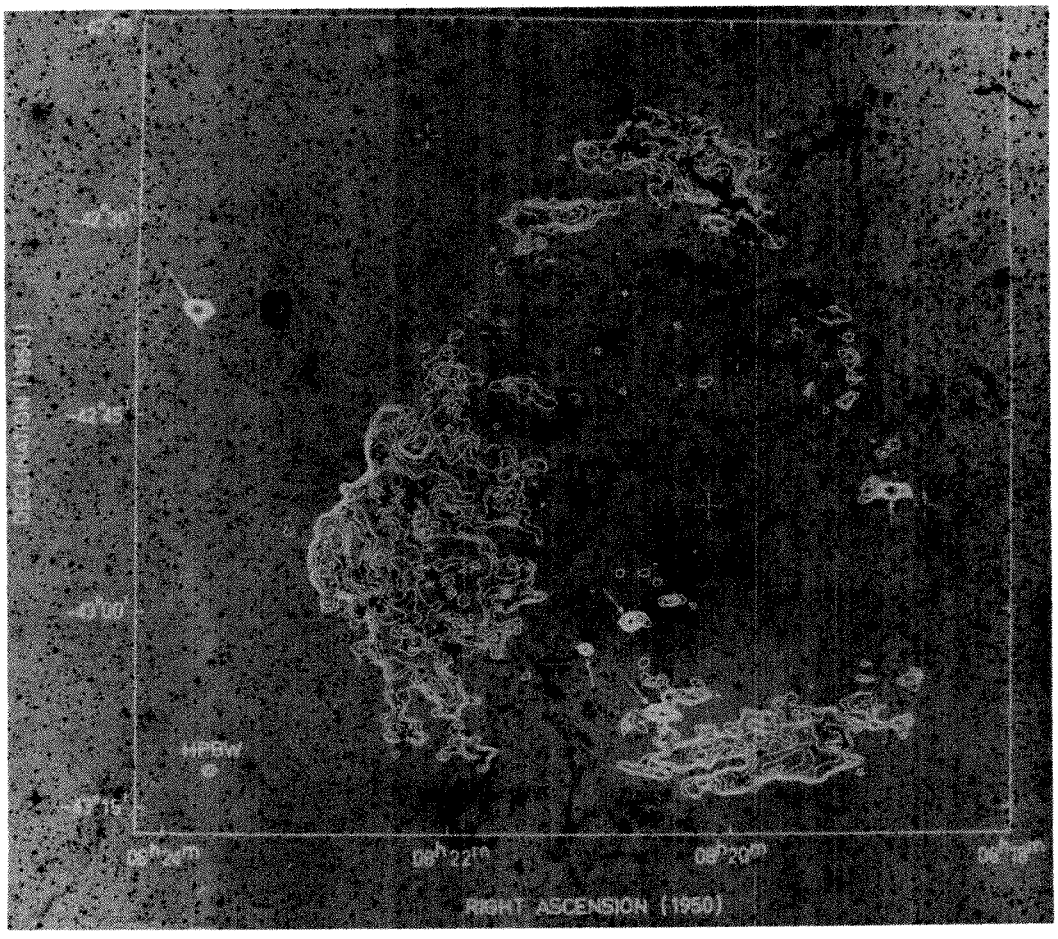


Fig. 1 Radio contours for Puppis A (ref. 2) superimposed on a deep Hα + [N II] plate from the UK Schmidt Telescope[1]. The swirl feature is visible as a group of faint filaments just east of the apparent centre. The 3 × 5 arcmin field for our CCD frames (Fig. 2) is indicated by the corners, and the expansion centre for the oxygen-rich filaments[10] is shown by the black ellipse 3 arcmin to the north-west.

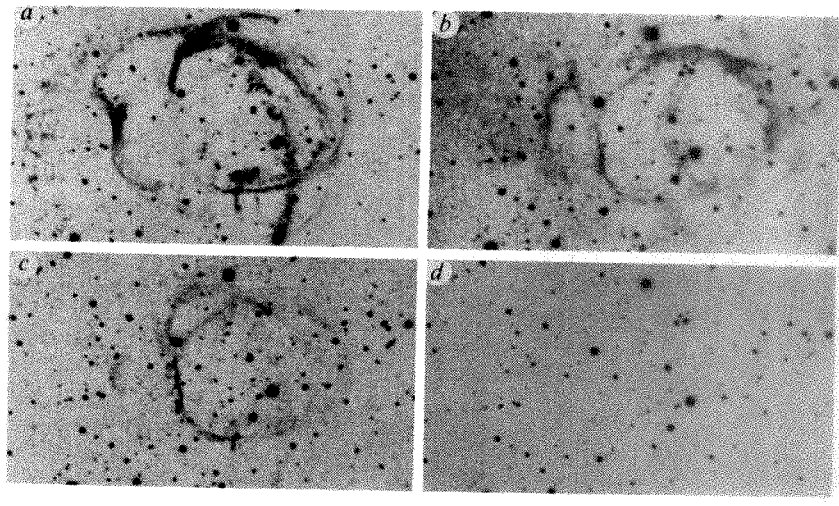


Fig. 2 Narrow-band CCD images of the Puppis A swirl region taken from the CTIO 4-m telescope in the light of Hα + [N II] (*a*), [O III] (*b*), [S II] (*c*), and a 6,100-Å continuum band (*d*). The field is 3 × 5 arcmin; north is up and east is to the left.

single long-slit spectrum with an exposure time of 2,400 s at a position chosen to cross the brightest filaments on the [O III] and Hα + [N II] images. The spectra (Fig. 3) from these filaments are clearly different from those of shock-heated interstellar material and indicate high concentrations of heavy elements. The oxygen filament (Fig. 3*a*) shows strong lines of [O I] and [O II] in addition to [O III], all at a radial velocity of -760 km s^{-1}. Lines of [N II], [Ne III] and faint Balmer lines all have the same radial velocity within the uncertainty of 50 km s^{-1}, which corresponds to about one quarter of the pixel width at 6,000 Å. This spectrum is similar to those of the 'omega' filament, of other fast, oxygen-rich knots in Puppis A (ref. 9), and of oxygen-rich filaments in young SNRs such as Cas A (ref. 11).

Table 1 CCD frames of Puppis A swirl

Ion	λ_c (Å)	$\Delta\lambda$ (Å)	Exposure (s)
[O III]	5,010	50	1,200
Hα + [N II]	6,560	60	600
[S II]	6,720	50	600
Continuum	6,100	150	300

Images taken using the CTIO 4-m telescope, 17 February 1988.

The spectrum of a bright filament from the Hα + [N II] image (Fig. 3*b*) shows that most of the emission is in the [N II] lines: the flux in [N II] 6583 is seven times that in Hα. Although such high [N II]/Hα ratios are quite rare in SNRs, they have been observed previously in bright filaments of Puppis A[4–6]. Nitrogen-rich material could have resulted from pre-supernova mass loss, similar to material in the quasi-stationary flocculi of Cas A (ref. 11) or as observed surrounding SN1987A (ref. 12). What is most unusual in the spectrum of Fig. 3*b* is a high velocity (-990 km s^{-1}) in a nitrogen-dominated filament. This may be a less extreme example of filaments such as the recently discovered 'fast-moving flocculi' in Cas A (ref. 13) which have been interpreted as material from the outer envelope of a Wolf-Rayet progenitor, ejected in the supernova event.

Another set of filaments in the swirl region has a flux in [S II] up to four times higher than in Hα + [N II], and ten times higher than in [O III], as measured on the PFCCD frames of Fig. 2. The strongest [S II] emission lies along an arc which continues to the north as a bright Hα + [N II] filament. No spectra have yet been obtained to confirm that these features are [S II] filaments, but the alternative interpretation, that they are red-shifted Hα + [N II], would require a radial velocity in excess of 5,000 km s^{-1}, over three times higher than any yet measured in Puppis A.

The swirl region comprises the most extraordinary confluence of filaments anywhere in Puppis A, and among known SNRs it is matched only by Cas A in its variety of chemically peculiar debris. If these filaments are part of the Puppis A remnant, some highly unusual mechanism must be proposed to account for their unique morphology, chemistry and velocities. For example, we might be seeing an aneurysm of ejecta, which as it moves outward is confined to a low-density tunnel pointed in our direction, rather like a rifle barrel.

We wish, however, to suggest a more radical interpretation of the data, namely that the swirl region is the remnant of a second supernova which has taken place inside the Puppis A cavity more recently than the event that resulted in Puppis A itself. The evidence for a second supernova consists of the appearance of the optical filaments, the lack of corresponding radio or X-ray features, the unusual chemical composition and the timescale for the swirl expansion.

The morphology of the swirl region is that of a filamentary shell; in particular, the combination of [O III] and [S II] together form an almost complete elliptical shell $\sim 100'' \times 125''$ in size. The absence of associated radio emission rules out the possibility that this shell is simply a chance alignment of a background SNR. The surface brightness of the swirl region is certainly no higher than that of Puppis A itself[2], so its intrinsic diameter should, according to the Σ–D relation, be no smaller. This would require a distance >50 kpc, which is clearly unreasonable despite the uncertainty that attends Σ–D distances.

If the small shell is indeed an SNR, then its surroundings must be extremely unusual to obscure its presence both in the radio and in X-rays. The hot cavity inside an older SNR shell, like that of Puppis A, would provide a high-pressure, low-density environment which could render a second supernova remnant within the cavity undetectable at radio and X-ray frequencies. Instead of leading to a strong shock with associated radio and X-ray emission, most of the kinetic energy from the second supernova would probably re-energize the existing bubble[14]. A related model of a supernova within a cavity has been proposed to explain the X-ray morphology of the young SNR N132D in the Large Magellanic Cloud (LMC)[15]. Optical emission could result if dense knots of ejecta from the second supernova are heated conductively by the surrounding hot plasma. Conduction heating is especially important in metal-rich material, and the spectral characteristics would mimic those of shock excitation[16].

The chemical composition and kinematics of the swirl structure are both indicative of a very young remnant. Its varied, metal-rich filaments are similar to those of Cas A, and the velocities are similar to those measured in other young, oxygen-rich SNRs such as N132D (ref. 17) and 0540-69 (ref. 18) in the LMC, and G292.0 + 1.8 (ref. 19) in our Galaxy. At the distance of Puppis A (2 kpc), the swirl radius of 1 arcmin is equivalent to 0.6 pc, and we have observed radial velocities >750 km s^{-1}

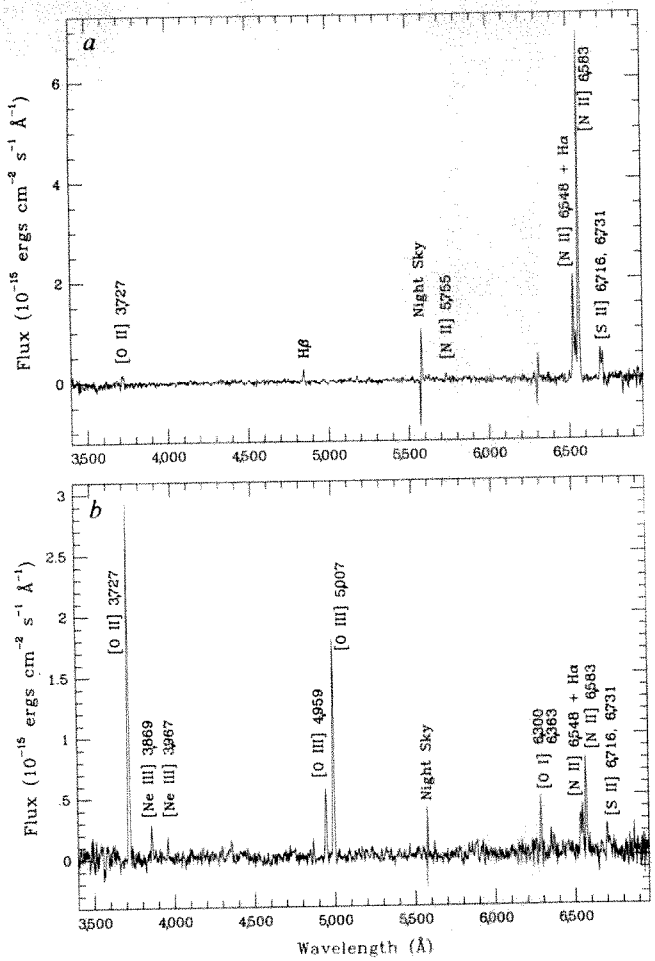


Fig. 3 Spectra of two knots from the same long-slit spectrum taken with the 2-D Frutti on the CTIO 4-m telescope. *a*, The brightest filament in the Hα + [N II] image is radiating primarily in [N II] at −990 km s^{-1}; [S II] is present at the same velocity, and all the oxygen lines are extremely weak. *b*, The brightest knot in the [O III] image shows lines of [O I], [O II] and [O III], all at a radial velocity of −760 km s^{-1}.

for material near the rim. Assuming a comparable transverse velocity, we obtain a timescale of <800 yr for the swirl structure to expand to its present size. By contrast, the kinematic age for Puppis A based on proper motions of the oxygen-rich filaments[9] is 3,700 yr, a value which is in reasonable agreement with age estimates based on the X-ray data.

Kinematic measurements provide potential tests for the 'second supernova' model. At 2 kpc a transverse velocity of 750 km s^{-1} would result in a proper motion of 0.08 arcsec yr^{-1}, so the timescale for the swirl expansion might be measurable within a few years. Meanwhile, more complete radial velocity data may reveal whether the swirl structure is made up entirely of (blue-shifted) material on the near side of the remnant, or whether it is indeed expanding.

Although the 'second supernova' model is speculative, it is interesting enough to merit conjecture about scenarios which could lead to two supernova events in rapid succession. One exotic possibility is that Puppis A was formed from a binary system in which the two components evolved in tandem to explode as near-simultaneous supernovae. The presence of filaments composed largely of oxygen and of oxygen-burning products indicates that Puppis A is the remnant of one or more highly evolved, massive stars, and the nitrogen-rich material suggests a Wolf-Rayet star in particular. Two nearly equal-mass stars in a massive binary system might evolve in tandem with the exchange and loss of mass until the originally more massive one becomes a Wolf-Rayet star and then explodes as a supernova[20–23]. Mass gain by the original secondary would accelerate its evolution until it too becomes a supernova. If the masses are close enough, both components should explode as supernovae within a time interval that is short compared with the evolutionary timescale, possibly producing a remnant such as we observe in Puppis A. Although common models of massive binary systems[24,25] typically assume an initial mass ratio of ~2.5:1 (so that the evolution of the two components proceeds at rates sufficiently different to produce long-lived X-ray binaries), components of nearly equal masses represent the most common configuration observed in unevolved massive binaries[26]. Systems that are balanced delicately enough for both components to explode within a few thousand years would be rare, but possible. Regardless of how they might have occurred, two supernovae within a single shell could comprise the beginning of a supershell, such has been proposed[27–29] to explain the complex structure of the interstellar medium.

The swirl region in Puppis A is comprised of filaments that are spectroscopically, and probably chemically, more diverse than those in any other SNR except Cas A. All the optical properties—irregular shell morphology, peculiar chemistry and relatively high velocities—are characteristic of a young SNR, but the absence of significant radio or X-ray emission is most uncharacteristic. Although the swirl region may be an extraordinary feature of the well-known remnant, we prefer the suggestion that it signifies a second supernova inside Puppis A. Further spectroscopic observations, to investigate the chemical compositions and radial velocities of several filaments, are needed to confirm this interpretation. Proper motion measurements leading to a kinematic age for the swirl structure far younger than that of Puppis A itself might provide a definitive test.

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A sub-millimetre aperture synthesis array for nonsolar planet detection

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The detection of Earth-like planets orbiting other stars has great scientific interest, but is a challenging observational goal. At wavelengths shorter than 4 μm a terrestrial planet would be about 10^{10} times fainter than a solar-type star, and at a distance of 10 parsecs the planet–star separation would never exceed 0.1 arcsec. At wavelengths longer than 50–100 μm the brightness ratio is reduced by four to five orders of magnitude. Here we consider the possibility of using aperture synthesis to image other planetary systems at sub-millimetre wavelengths (50–1,000 μm). This approach requires only a small increase in dynamic range over that already demonstrated by the Very Large Array at longer wavelengths.

The concepts described here are part of a continuing study to determine which observational techniques could directly detect terrestrial and larger planets orbiting a star within 10 pc of the Sun, using no more than 24 h of observing time per star. This is a considerable challenge at any observing wavelength, but the nature of the challenge differs from radio to infrared and visible wavelengths. At radio wavelengths the primary challenge is obtaining sufficient sensitivity, whereas at infrared and visible wavelengths the primary challenge is obtaining sufficient dynamic range (that is, by suppressing instrumental sidelobes and scattered light for a direct imaging system, or nulling the central star for an infrared or visible interferometer[1–3]).

Using wavelengths longer than ~50 μm has the advantage that the ratio of thermal flux density from a solar-type star and a terrestrial-type planet is orders of magnitude smaller than at shorter wavelengths. In fact, the expected flux density ratio ($\sim 3\times10^{5}$) is only a few times the dynamic range of radio maps currently produced by the Very Large Array (VLA), suggesting that straightforward aperture synthesis could produce images of other planetary systems. At long (radio) wavelengths, however, the absolute flux density of both star and planet decreases rapidly with increasing wavelength, so the region near the peak of the planet's black-body emission should be optimal. Figure 1 shows the spectrum of a terrestrial planet and solar-type star. This letter considers the design of an aperture synthesis imaging instrument operating in this wavelength range (between 50 and 1,000 μm).

The required instrument characteristics are an angular resolution of <0.05 arcsec, a total field of view >2 × 2 arcsec, sufficiently high sensitivity to detect a terrestrial planet at 10 pc in 1 day, and a dynamic range sufficient to prevent sidelobes of the star from hiding a planet~0.1 arcsec away. The ability to image the entire field of interest with full sensitivity at every pixel, and thus to map out the positions of all planets at once, is clearly important as we do not know how complex other planetary systems may be. Resolving planetary disks at such distances is not practical; for example, the angular size of the Earth at 10 pc is only 1 microarcsec.

The resolution and field of view requirements listed above imply baselines from 0.1λ to at least 4λ metres in length, where λ is the observing wavelength in μm. Thus a range of baseline spacings of at least 40 to 1 must be sampled by the array. The field of view also sets an upper limit of 0.1λ metres for the diameter of each individual telescope (in order that the primary beams will not be too small). Otherwise many observations of the region around a target star would be required, greatly reducing the efficiency of the search.

An additional limit imposed by the field of view is the maximum fractional bandwidth. For a heterodyne interferometer it is useful to consider bandwidth in terms of frequency rather than wavelength. The relative response, R, to a source at an angular distance r from the centre of the field is

$$R = \frac{\sin(\pi \Delta\nu\, r/\nu\theta)}{(\pi \Delta\nu\, r/\nu\theta)}$$

where $\Delta\nu$ is the bandwidth, ν is the centre frequency of the observed radiofrequency band, and θ is the width of the synthesized beam. All sources not at the field centre will be radially elongated by $r\,\Delta\nu/\nu$. Preventing significant loss of sensitivity at the field edge requires that $\Delta\nu/\nu \ll \theta/r$. With $r = 2$ arcsec and a synthesized beam smaller than 0.05 arcsec, we need $\Delta\nu/\nu \ll 0.025$. For a multichannel system this limit applies to each individual intermediate frequency channel, not to the combined bandwidth.

The r.m.s. noise (S_n) in an image produced by aperture synthesis with all data weighted equally is given by

$$S_n = \frac{2^{1/2}kT_s}{\eta A[\tau\,\Delta\nu(N(N-1)/2)]^{1/2}}\ \mathrm{W\,m^{-2}\,Hz^{-1}}$$

where the Boltzmann constant $k = 1.38\times10^{-23}$ W Hz^{-1} K^{-1}, T_s is the system temperature, η is the aperture efficiency of the telescopes, A is the physical aperture of a single telescope, τ is the total integration time in seconds and N is the number of individual telescopes in the array.

The minimum total aperture of the array has been calculated assuming telescope efficiencies of 50%, system temperatures of $h\nu/k$ or 50 K, whichever was higher, a total IF bandwidth of 300 GHz (10% of the observing radiofrequency at λ = 100 μm), a total integration time of 1 day, and the use of global fringe fitting[4] (see below). The large IF bandwidth could be produced by combining a large number of parallel channels, but the low system temperature will require significant advances in sub-millimetre mixer or amplifier technology.

The flux density S_p of a planet at temperature T subtending a solid angle of Ω steradians is

$$S_p = \frac{2h\nu^3\Omega}{c^2(e^{h\nu/kT}-1)}\ \mathrm{W\,m^{-2}\,Hz^{-1}}$$

For a terrestrial planet at 10 pc, $S_p \approx 7.3\times10^{-34}$ W m^{-2} Hz^{-1} = 7.3×10^{-8} Jy at 100 μm. We would normally require $S_p/S_n \geq 5$, but if global fringe fitting is used the minimum useful signal-to-noise ratio can be decreased by an additional factor[5] of $\sim\sqrt{N}$.

With these assumptions, the sensitivity requirement implies a total physical aperture NA (collecting area) of $\sim\lambda^2$ m^2, where

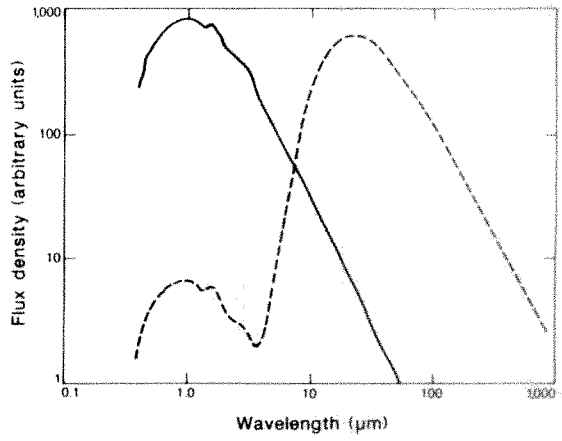


Fig. 1 The flux density of a terrestial planet (dashed line) and a solar-type star (solid line) plotted against wavelength. The curve for the star has been multiplied by 10^{-8}. At wavelengths shorter than ~4 μm the planet flux density comes from reflected starlight; at longer wavelengths it corresponds to thermal black-body emission.

λ in μm. This applies for wavelengths longer than ~300 μm. Because the collecting area of an individual telescope is proportional to λ^2 for a constant beamwidth, the total number of telescopes in the array remains constant ($N \approx 100$ in this example) for wavelengths longer than ~300 μm.

At shorter wavelengths the system temperature increases because of quantum effects ($T_{sys} \approx h\nu/k$), so the required collecting area decreases more slowly with decreasing wavelength. For wavelengths between ~50–300 μm the total aperture of the array is ~300 λ m^2. At wavelengths shorter than ~20 μm the flux density of the planet turns over and the required aperture increases rapidly.

There is a minimum for the total aperture of ~10^4 m^2 at λ = 20–30 μm. Between ~9 and 100 μm the required aperture is within a factor of three of the minimum. This minimum is about 0.7 times the collecting area of the VLA. The detection of planets similar to Jupiter is much easier. Even with only one tenth as much total bandwidth (30 GHz), Jupiter could be detected at 10 pc in 24 hours with a total aperture of ~2×10^3 m^2 in the 20–50 μm region. This is only 15% of the VLA collecting area. Figure 2*a* shows the minimum collecting area as a function of wavelength for both terrestrial and Jupiter-type planets.

Figure 2*b* shows the number of individual telescopes needed for the array as a function of wavelength. At each wavelength, the telescopes are assumed to be as large as the field-of-view requirement allows. This figure makes it clear that the 20–50 μm spectral region is not desirable, despite the fact that it requires the smallest total collecting area. It may be more practical to use a wavelength closer to 300 μm to minimize the total number of telescopes rather than the total collecting area.

All of our sensitivity calculations assume that the total number of telescopes (N) in the array is large (given by the number of 0.1λ-metre diameter telescopes needed to reach the total required collecting area). For example, at λ = 100 μm the maximum individual telescope diameter is 10 m and the total number of telescopes required to detect a terrestrial planet in 1 day is ~250. For a Jupiter-like planet the number of telescopes required is ~40, assuming a 1% bandwidth instead of 10%. There are several advantages to having a large N, including more accurate self-calibration of the individual telescope amplitudes and phases and, as mentioned before, an increase in the total sensitivity of the array by a factor of ~$\sqrt{N}$ through global fringe fitting. However, if a smaller N is desired, the number of IF channels (and thus the total bandwidth) or the total observing time can be increased. As sensitivity is proportional to $\sqrt{\tau \Delta\nu}$ a large increase in τ or $\Delta\nu$ is required to significantly reduce the total collecting area. The maximum integration time is limited by the orbital motion of the planet around its parent star. In the case of a face-on planetary system, a terrestrial planet orbiting a star 10 pc away will move by half the synthesized-beam width in 2 weeks. Thus, if we are willing to accept some smearing of the planet image, the required number of telescopes (or total collecting area) could be reduced from the values given above by about a factor of four, at the expense of observing time.

The individual telescopes must not be so small that fringes cannot be detected on at least one baseline to each telescope. This suggests a hybrid array composed of many telescopes whose size is no larger than that set by the field-of-view requirement, along with one larger telescope whose primary beam may be too small to include any planets. The purpose of this single large telescope is to provide a more sensitive baseline to each of the other telescopes, so that global fringe fitting may be used. As described in detail by Schwab and Cotton[4], global fringe fitting will then be able to detect the weaker fringes on all baselines between the small telescopes. This technique has come into use recently for very-long-baseline interferometry (VLBI) fringe searching.

The relatively large flux density of the star is an advantage here, as it allows the array to be phased up (focused) nearly continuously. By adjusting the individual telescope phases to keep the measured visibility phase from the star equal to zero on every baseline, the much weaker fringes from any planets will be nearly 'stopped' as well. This allows long coherent integrations.

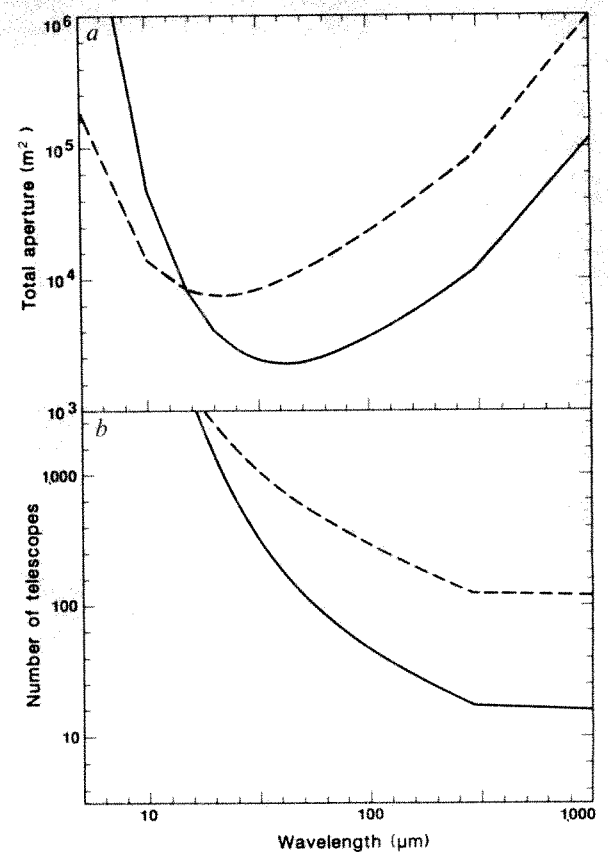


Fig. 2 Minimum total collecting area (*a*) and number of telescopes (*b*) for an aperture synthesis array designed to detect terrestrial planets at a distance of 10 pc in 24 hours. *a*, The dashed curve assumes a 300-GHz bandwidth, 50% aperture efficiency and a system temperature which is the higher of $h\nu/k$ or 50 K. Using wavelengths near 20–30 μm minimizes the required total physical size of the array. The solid curve shows the total aperture required to detect a gas giant planet similar to Jupiter, with the same assumptions as before except for a 30-GHz bandwidth. *b*, Number of telescopes required to provide the array collecting area. As above, the dashed curve is for a terrestrial planet and the solid curve is for a Jupiter-like planet. For wavelengths longer than ~300 μm the numer of telescopes is nearly constant, whereas for shorter wavelengths the number increases rapidly. Note that for the Jupiter-like planet only about 16 telescopes are needed at long wavelengths, assuming a bandwidth of 30 GHz.

The dynamic-range requirement implies that the array must have many separate baselines, and thus a large N, to provide dense and uniform coverage of the aperture (u, v) spectral plane. In addition, the (u, v) coverage should be 'centrally condensed'—that is, there should be more short baselines than long ones. This tends to reduce sidelobe levels in a manner similar to apodization in optical systems. A further requirement for very-high-dynamic-range-aperture synthesis mapping is an absence of errors that are baseline-dependent rather than telescope-dependent. This will require careful attention to polarization response, bandpass response and correlator design.

If we assume that the zodiacal light has a uniform surface brightness within the field of view, then its only effect is to increase the interferometer system temperature and thus the level of thermal noise in the images. The same is true of thermal emission from the telescope mirrors if the temperature remains constant. Both noise sources, if constant, are negligible com-

pared with the heterodyne receiver quantum noise. They may cause problems if spatial structure in the zodiacal light or telescope temperature fluctuations cause systematic variations in apparent fringe amplitude.

The zodiacal light surface brightness is given by

$$B_\nu = \frac{2h\nu^3\varepsilon}{c^2(e^{h\nu/kT}-1)} \text{ W m}^{-2}\text{ Hz}^{-1}\text{ ster}^{-1}$$

For an emissivity ε of 3×10^{-7}, a temperature of 240 K and a wavelength of 100 μm, the surface brightness within a 2 arcsec field of view is about 5% of the flux density of a solar-type star at 10 pc. Thus, fluctuations in zodiacal light at the level of 1 part in 10^4 over sub-arcsecond angular scales, if present, must be discriminated from true planetary signals. Space-based visible or infrared mapping of selected regions of the zodiacal background with sub-arcsecond resolution would constitute a valuable precursor mission.

This project requires that a large number of small telescopes be deployed, which means that the cost per telescope must be small. In addition, the cross-correlation processing load will be very large. The correlator must be able to handle an extremely high data rate ($>10^{14}$ bits per second) because of the large number of baselines $N(N-1)/2$ and large bandwidth (many GHz). For comparison, the digital correlator at the VLA handles 351 baselines and a total bandwidth of 200 MHz. There has been considerable recent progress in the design of large digital correlators, particularly in the development of the FX approach, in which the data from each telescope are Fourier-transformed to the frequency domain before cross-multiplication with all other spectra, which has made very large correlators more practical. Optical cross-correlation is a possibility, although baseline-dependent errors may be more difficult to control with an optical correlator.

A large interferometer array of this sort would be able to meet all of the goals listed above. It would also be an immensely valuable instrument for other areas of astronomical research. Studies of compact extragalactic radio sources, microwave background fluctuations, radio luminosity functions and source counts to very low flux densities are a few obvious examples. The practicality of this approach to nonsolar planet detection is dependent on financial, not basic technical, considerations.

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Probable detection of organic-dust-borne aromatic $C_3H_3^+$ ions in the coma of comet Halley

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The heavy-ion analyser PICCA[1,2] on the Giotto spacecraft was used to determine the composition and energy distribution of positively charged ions in the coma of comet Halley. Here we argue that the distinct peak observed at mass 39 AMU is due to the aromatic cation $C_3H_3^+$ (cyclopropenyl). Laboratory mass spectroscopy of unsaturated hydrocarbon-chain molecules and aromatics yields large intensities of mass-to-charge ratio (m/z) 39; the spectra of the aromatic molecules also indicate the presence of $C_4H_3^+$ ($m/z=51$) and $C_5H_5^+$ ($m/z=65$). These latter products are not seen in the PICCA data, suggesting that in the cometary environment unsaturated hydrocarbon chains predominate over aromatics. We suggest that the progenitor of the hydrocarbon chains (which are apparently the parents of the $C_3H_3^+$ ion) are the 'CHON' dust particles observed around the comet[3]; the $C_3H_3^+$ ion may also be one of the parent molecules of C_3, which is a common feature of cometary spectra[4], although its origin has remained obscure.

The PICCA (positive-ion-cluster-composition analyser) instrument uses an electrostatic analyser and measures the ratio of energy to charge (E/z). It takes advantage of the large relative fly-by velocity and the fact that cometary ions in the inner coma have very low temperatures (thermal velocity ≪ ram velocity) and are invariably singly charged. Thus the measurements of E/z are, to a very good approximation, proportional to the mass. The mass resolution is $\Delta m = 0.4$ AMU (atomic mass units) in the mass range 10–50 AMU, and $\Delta m = 1$ AMU in the mass range 51–210 AMU. Ions were detected by two channel electron multipliers with different geometric factors to provide a large dynamic range. The spectrum integration time of 3.2 s is different from the spacecraft spin period of 4 s.

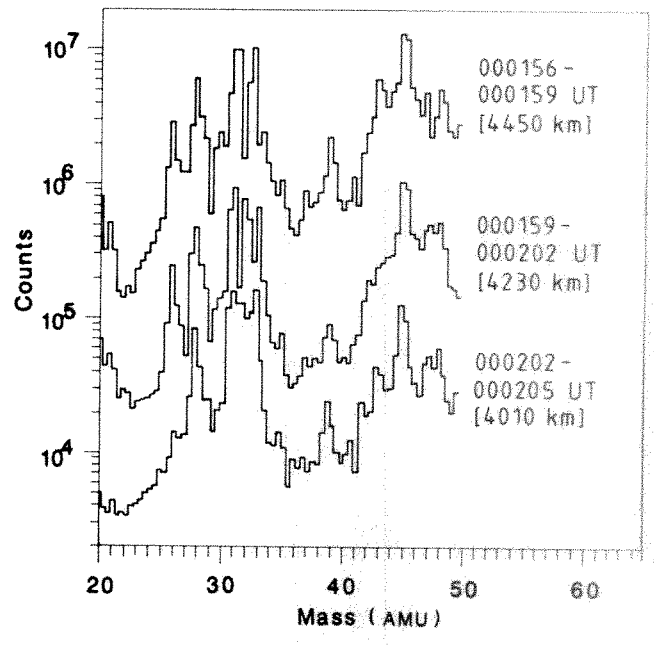


Fig. 1 Three consecutive measurements of mass spectra. The time is given in spacecraft event time and corresponds to cometocentric distances of (top to bottom) 4,450, 4,230 and 4,010 km.

In the following, we show spectra from the PICCA instrument

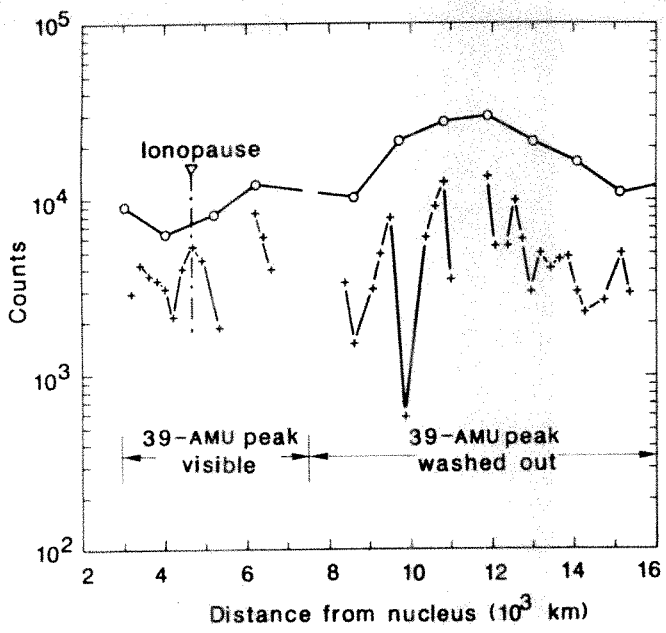


Fig. 2 Radial dependence of the mass channels 39 AMU (+) and 63–73 AMU (○). The intensity, given in counts s^{-1}, is proportional to the density. The position of the ionopause is indicated.

inside the cometary ionopause. The ionopause (which is a tangential discontinuity surface) is the outer boundary of the magnetic-cavity region detected at a cometocentric distance of ~4,600 km during Giotto's inbound passage[5]. In this region, the ion temperatures are as low as 300 K (ref. 6). In this paper, we will discuss peaks in the spectra at masses of 37, 39 and 41 AMU. Although the PICCA instrument measured ions up to 105 AMU, only the part of the spectra close to the peaks of interest are presented here.

In Fig. 1, three consecutive spectra are shown, displaced vertically for clarity. The time is given in spacecraft event time for each spectrum and corresponds to a certain cometocentric distance. The distance from the comet varies from ~4,450 km to ~4,010 km. The count rates correspond to the central spectrum and are proportional to the density. The two other spectra are shifted up and down by half an order of magnitude relative to the central one. The peak at mass 39 AMU which is visible in all three of the displayed spectra can be observed continuously up to cometocentric distances of ~7,500 km with no dramatic change at the ionopause. Outside the ionopause, the thermal-energy spread of the ions limits the effective mass resolution of the instrument so that the peaks get washed out. The transition from sharp to washed-out is not accompanied by a pronounced drop in the count rate (density).

Figure 2 shows the radial dependence of the 39-AMU peak intensity, which is proportional to the density. The density of the 39-AMU species inside the ionopause at ~4,200 km is ~5 ions per cm^3. The values of the intensity for a cometocentric distance $r > 7{,}500$ km are obtained by the fitting process described by Mitchell *et al.*[7]. For comparison, a solid line trace is given which shows the count rates summed from the channels 64–73 AMU (inclusive) and averaged over 16 s. Both curves show similar features, including an intensity maximum between 11,000 and 12,000 km. Unfortunately, it is not possible to deduce a reliable radial profile because of the spin modulation of the instrument and data gaps.

The unambiguous identification of a given peak in the spectra shown in Fig. 1 with a particular ion is not possible. In Table 1, however, we have listed the dominant ion species that are expected from a CHON-dominated medium[8]. As pointed out earlier, we take $z = 1$ (singly charged ions). The PICCA data

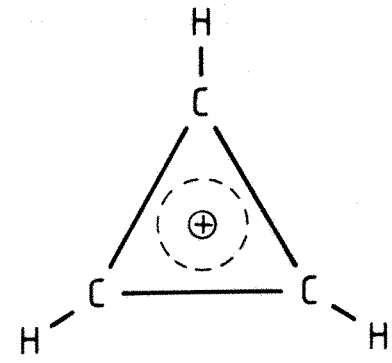


Fig. 3 The structure of $C_3H_3^+$, the cyclopropenyl cation.

show a strong preference for odd mass numbers over even mass numbers; this distribution is typical for a nitrogen-poor non-radical ion mixture. Radical ion species are thus unlikely and are shown in parentheses in Table 1.

The most likely identification of the $m/z = 39$ feature is as the aromatic $C_3H_3^+$ (cyclopropenyl) rather than as a hydrocarbon-chain molecule (see below). The cyclopropenyl ion has a ring structure and shows an aromatic character, having an appropriate number of π electrons (Fig. 3). It has no permanent electric dipole moment and will not be visible in the microwave spectrum.

Although aromatics have a highly unsaturated character, they do not enter into addition reactions and are therefore very stable. Besides $C_3H_3^+$ there are only a few other stable cations with aromatic character. For example $C_7H_7^+$, tropyl ($m/z = 91$), is possibly one of the ions that we identified in the PICCA instrument outside the ionopause.

Inside the ionopause individual mass peaks have been resolved below 70 AMU, whereas outside the ionopause a number of broad mass groups have been observed, one of which is centred at 90 AMU (ref. 9). Contributions to this broad mass group need not come only from an ion of $m/z = 90$, but may also be from ions of neighbouring masses ($m/z = 89$ (dehydrotropyl) and $m/z = 91$ (tropyl)). Although Mitchell *et al.*[9] suggest that $m/z = 90$ corresponds to the ion $HOCH_2CH_2COOH^+$, observed in laboratory residues resulting from simulation experiments[10], $m/z = 91$ could correspond to $C_7H_7^+$, which is known to be very abundant and stable. Recently, Giard *et al.*[11] made the first identification of polycyclic aromatic hydrocarbon molecules in the diffuse emission of the galactic disk.

Laboratory mass spectroscopy of the destruction products of unsaturated hydrocarbon-chain molecules and aromatics shows a pronounced peak at $m/z = 39$ (refs 12, 13). Aromatics also commonly show products at $m/z = 51$ and $C_4H_3^+$ 65. These products, which could be identified as $C_4H_3^+$ and $C_5H_5^+$, are not seen abundantly in the PICCA data. On the other hand, unsaturated hydrocarbon-chain molecules or compounds with oxygen do not show abundant peaks at the above values but instead show peaks at $m/z = 41$ (possibly the cyclopropanyl ion, $C_3H_5^+$) and at $m/z = 43$. The PICCA instrument sees these latter ions rather abundantly. We therefore conclude that the main progenitor of the $m/z = 39$ peak is a long unsaturated chain

Table 1 Possible identification of mass peaks in a CHON-dominated medium

m/z	Ion species
37	C_3H^+, $H^+(H_2O)_2$
39	$C_3H_3^+$, $(C_2NH^{+\cdot})$
41	$C_3H_5^+$, C_2HO^+, $(C_2H_3N^{+\cdot})$

Radicals are enclosed in parentheses.

molecule, although aromatic progenitors are not entirely excluded.

As indicated in Table 1, the 39-AMU mass peak could also be produced by the molecule $C_2NH^{+\cdot}$, assuming only CHON as building blocks. This molecule is a radical, however, and no indications of other homologous radical ions are found in the spectra. Moreover, its reactivity, relative to $C_3H_3^+$, is much higher and therefore there is a high probability of reactions with H_2O, which is the most dominant molecule in the inner coma. The possibility that $m/z = 39$ could also correspond to potassium is ruled out because there is far too little K^+ in the dust to produce such an abundant yield of gas-phase K^+ (ref. 14).

A further decomposition of mass 39 by dehydrogenation to C_3H^+ (mass 37, which is also found, to a lesser extent, in the PICCA spectra (see Fig. 1)) is only possible in high-energy collision processes. These are not likely in an inner cometary coma, where the plasma is very cold ($\leq$300 K). But we note that a significant contribution to the mass-37 peak could also come from $H_5O_2^+$ by the reaction:

$$(H_2O)_2^+ + H_2O \rightarrow H_5O_2^+ + OH$$

if a substantial fraction of water sublimating from the nucleus is in the form of the dimer $(H_2O)_2$ (ref. 15).

We now consider the origin of $C_3H_3^+$. Is it derived from the dust component or from some gas component emanating from the nucleus?. Assuming that the ion of mass 39 participates readily in the atmospheric chemistry, the required abundance of the progenitor molecule can be estimated. The primary gas-phase destruction processes in the cometary atmosphere for any given ion are ion-molecule reactions with H_2O, proceeding at a typical rate $\tau_{im} \approx 10^{-9}\, n_{H_2O}\ s^{-1}$, and recombination, with a rate $\tau_{rec} \approx 10^{-6}\,(300/T_e)^{1/2}\, n_e\ s^{-1}$ (ref. 16). Using a number density for H_2O of $n_{H_2O} = 5\times10^6\ cm^{-3}$ (ref. 17), electron temperature $T_e = 100$ K and electron number density $n_e = 1{,}500\ cm^{-3}$ (from model calculations)[18] at a distance of $3\text{–}4\times10^3$ km, $\tau_{im} = 5\times10^{-3}\ s^{-1}$ and $\tau_{rec} = 2.5\times10^{-3}\ s^{-1}$. This implies that the ion $m/z = 39$ ion must be produced at a rate of $\sim 7\times10^{-3}\, n_{39^+}\ cm^{-3}\ s^{-1}$ (where n_{39^+} is the number density of the ion of mass 39 and $\tau_{rec} + \tau_{im} \equiv \tau_d \approx 7\times10^{-3}\ s^{-1}$ is the total destruction rate).

The production of the $m/z = 39$ ion may occur through either (1) ion-molecule reactions ($X^+ + Y \rightarrow X_{39}^+ + $ products); (2) photoionization of a neutral ($X + h\nu \rightarrow X_{39}^+ + $ products); or (3) photodissociation of an ion ($X^+ + h\nu \rightarrow X_{39}^+ + $ products). Clearly, (1) requires that Y be about as abundant as H_2O because X_{39}^+ is destroyed by H_2O by the analogous reaction. Reaction (2) requires n_x (the number density of X) to be equal to $n_{39^+}(\tau_d/\tau_{pi})$, where τ_{pi} is the photoionization rate. If $\tau_{pi} = 10^{-6}\ s^{-1}$ (a typical photoionization timescale) and $n_{39^+} = 5\ cm^{-3}$, then $n_x = 3.5\times10^4\ cm^{-3}$, which is a density comparable to that of the species, such as NH_3 and CH_4, that are the next most abundant after H_2O and CO. Again, n_x appears to be too large to remain unobserved. Reaction (3) requires $n_{X^+} = n_{39^+}(\tau_d/\alpha)$, where α is the photodissociation rate of X^+. To estimate α we consider the most steeply decreasing mass peak observed between 3,000 km and 4,000 km, which is the mass-75 group, and attribute the entire decrease to photo-processes. Assuming that the ion velocity inside the ionopause is equal to the neutral velocity (a good approximation because of the strong collisional coupling between ions and neutrals there) and taking the latter to be $\approx$0.8 km s^{-1} (ref. 19), we get $\alpha \leq 5\times10^{-4}\ s^{-1}$, and consequently, $n_{X^+} \geq 14\, n_{39^+}$. But the only observed mass peak in the PICCA data beyond mass 39 with a significantly higher intensity is that for $m/z = 45$ (which is $\sim$5 times higher). This is unlikely to produce the $m/z = 39$ ion through reaction (3).

Although the above gas-phase calculations are based on reaction rates that are somewhat uncertain, we believe that they are sufficiently reliable for our conclusions to be valid. Thus we can eliminate a gas-phase progenitor molecule for $C_3H_3^+$, so that the only alternative source that could be significant seems to be the observed circumnuclear CHON dust particles. This is not surprising, as dust has already been strongly implicated as a source of CO (which is the second most abundant species after H_2O, comprising 10–20% of the cometary atmosphere), as well as a source of CN.

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Estimation of the rate of volcanism on Venus from reaction rate measurements

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Maintenance of the global H_2SO_4 clouds on Venus requires volcanism to replenish SO_2, which is continually removed from the atmosphere by reaction with calcium minerals on the planet's surface[1–4]. Here we present laboratory rate data for the reaction between SO_2 and calcite ($CaCO_3$) to form anhydrite ($CaSO_4$). If this reaction rate represents the SO_2 reaction rate on Venus, then all SO_2 in the venusian atmosphere (and thus the clouds) will disappear in 1.9 Myr unless volcanism replenishes the lost SO_2. The required volcanism rate, which depends on the sulphur content of the erupted material, is in the range 0.4–11 km^3 of magma erupted per year. The Venus surface composition at the Venera 13, 14 and Vega 2 landing sites[5,6] implies a volcanism rate of approximately 1 $km^3\ yr^{-1}$. This geochemically estimated rate can be used to determine if either (or neither) of two discordant geophysically estimated rates (2 $km^3\ yr^{-1}$ versus 200–300 $km^3\ yr^{-1}$)[7–9] is correct. It also suggests that Venus may be less volcanically active than the Earth.

Calcite acts as a net sink for SO_2 on Venus by incorporating it into the crust through the net reaction[1–4]

$$CaCO_3\,(\text{calcite}) + SO_2 \rightarrow CaSO_4\,(\text{anhydrite}) + CO \qquad (1)$$

which proceeds to the right because the observed SO_2 abundance on Venus is $\sim$100 times higher than the value in equilibrium with coexisting calcite and anhydrite[4]. Calcite is also predicted by several authors to be present on the Venus surface because it appears to be capable of buffering the CO_2 abundance on Venus through the Urey reaction ($CaCO_3 + SiO_2 = CaSiO_3 + CO_2$)[10–13]. These predictions concerning possible roles for calcite on Venus, the exemplary nature of reaction (1), and the availability of high-purity $CaCO_3$ in several forms led us to study reaction (1) as an example of SO_2 removal processes. However, as

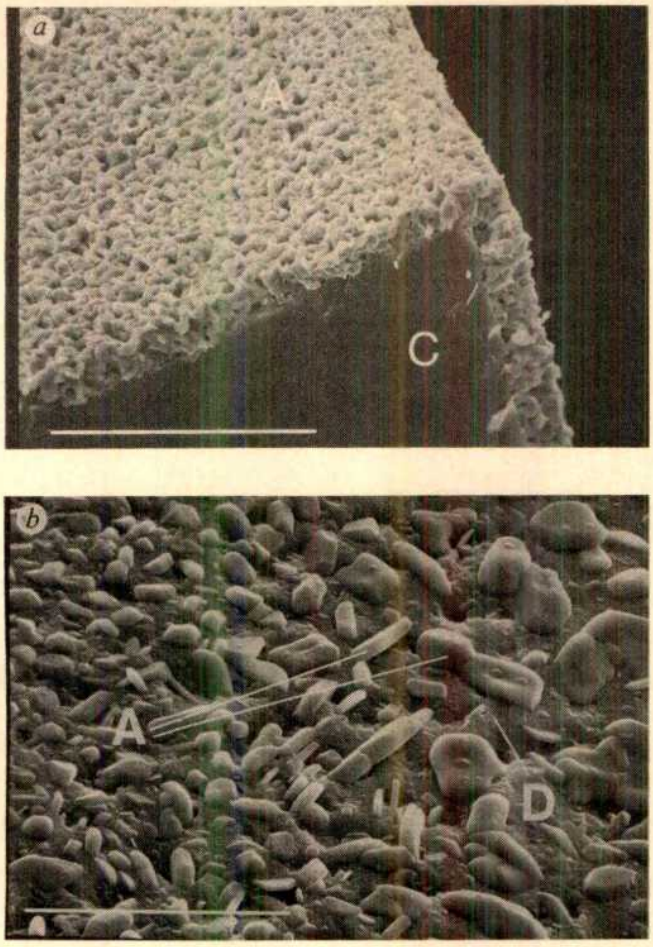


Fig. 1 Scanning electron micrographs of chemically reacted samples. *a*, Fracture surface, showing a $CaSO_4$ (anhydrite) layer (A) completely covering a partially reacted calcite crystal (C) (850 °C for 192 h) *b*, Top surface, showing $CaSO_4$ (A) growing on a partially reacted diopside crystal (D) (833 °C for 48 h). Scale bars indicate 100 μm (*a*) and 50 μm (*b*).

Fig. 1 illustrates, SO_2 also reacts with other calcium minerals (such as, diopside, $CaMgSi_2O_6$), which are believed to be present on the surface of Venus[14,15]. The kinetics for these alternative reactions are also being measured and will be reported later.

Reaction (1) was studied by heating clear calcite crystals (Iceland spar) of known impurity content (~350 p.p.m. total impurities from analysis by inductively coupled plasma emission spectroscopy), weight and surface area in SO_2-bearing gas streams for varying time periods at three different temperatures. Experiments were done at temperatures of 600–850 °C and ambient atmospheric pressures with SO_2–CO_2 gas mixtures nominally containing 1% (by volume) SO_2. Thus, the molecular number density of SO_2 is about the same as that at the surface of Venus, where the atmospheric pressure is ~100 times larger but the SO_2 volume mixing ratio is ~100 times smaller[16]. The mixtures also typically contained <1 p.p.m. O_2 and ≤10 p.p.m. H_2O as impurities. Experiments were also done with gas mixtures containing up to 100 p.p.m. O_2; however, the rates were independent of oxygen fugacity (and thus of the CO/CO_2 ratio) within this range of O_2 content. Standard techniques were used to monitor and control gas flow and temperature (to better than ±5 °C). The rate of reaction (1) was determined by three independent methods: (1) measuring the weight gain at the end of reaction, (2) using scanning electron microscopy (SEM) to measure the porosity and thickness of the reacted surface layers from micrographs of external and fracture surfaces (see, for example, Fig. 1), and (3) SO_4^{2-} analyses of reacted samples by ion chromatography. SEM examination of many samples (in combination with X-ray line scans and energy-dispersive spectroscopy (EDS) on the SEM) showed that $CaSO_4$ layers covered all external surfaces of the reacted samples. X-ray powder patterns of samples and standards confirmed that the layers were anhydrite (in particular, there was no evidence for calcium sulphite) and that unreacted calcite was the only other phase present in the samples. The ion chromatography also verified the absence of anion impurities at levels ≥10 p.p.m.

The experimental run conditions and rate data are summarized in Table 1 and Fig. 2. Rate data using the weight-gain, SEM and SO_4^{2-} analysis methods for the same sample agree within the combined experimental uncertainties (1σ) shown in Table 1. A weighted linear least-squares fit to the data gives a rate equation $R = 10^{19.64(\pm 0.28)} \exp(-15{,}248(\pm 2{,}970)/T)$ molecules $cm^{-2}\,s^{-1}$, with a corresponding activation energy of 126.8 ± 5.4 kJ mol^{-1}. The rate of reaction (1) was extrapolated downward to Venus surface temperatures (~660–750 K) using this equation and the Pioneer Venus radar-altimetry data[17] and atmospheric (P–T) profile[18] to take into account the altitude dependence of the rate. The derived global-mean SO_2 reaction rate is $\sim 4.6 \times 10^{10}$ molecules $cm^{-2}\,s^{-1}$, equivalent to ~1 μm of $CaSO_4$ being deposited per year. However, aeolian weathering[19] by the ~1.0-m s^{-1} winds on Venus[20] will probably remove this thin $CaSO_4$ layer on a shorter timescale, so the laboratory rate data for reaction (1) are applicable to Venus.

Chemical analyses of the Venus surface by the Venera 13, 14 and Vega 2 spacecraft[5,6] give a CaO content (by mass) of $7.90 \pm 0.51\%$ (weighted mean $\pm 1\sigma$); this is also taken as the areal percentage because of similar rock and oxide densities. Assuming that the experimental rate is representative of the rate at which SO_2 is depleted by reaction with calcium minerals on the Venus surface, the observed SO_2 column density (2.2×10^{23} molecules cm^{-2})[16] would be removed from the Venus atmosphere in $\sim 1.9 \times 10^6$ yr in the absence of a comparable sulphur source. However, maintenance of the global H_2SO_4 clouds, which are formed by the ultraviolet-sunlight-powered conver-

Table 1 Rate data used to calculate the activation energy

Sample	T (°C)*	Duration (hours)	Rate × 10^{-12} (molecules $cm^{-2}\,s^{-1}$)†
R44-1	602	95	1.67 ± 0.19‡
R45-1	602	314	0.85 ± 0.77
			1.05 ± 0.12‡
R45-3	602	314	1.01 ± 0.11‡
R45-4	602	314	1.20 ± 0.51
			0.68 ± 0.48§
R36-1	746	96	8.43 ± 2.93
			13.6 ± 6.0§
R36-2	746	96	23.2 ± 6.2
			19.9 ± 11.0§
R50-1	747	192	9.19 ± 1.60
R50-3	747	192	10.5 ± 7.8
R51-1	748	96	13.2 ± 4.0
R51-2	748	96	10.3 ± 5.6
R43-1	848	91	60.4 ± 6.8
			57.1 ± 31.7§
R43-2	848	91	53.2 ± 7.0
			67.2 ± 43.4§
R43-3	848	91	58.1 ± 7.0
			48.4 ± 12.1§

* The estimated temperature uncertainty is ±5 °C.

† The number of SO_2 molecules reacted per cm^2 surface area per second. The rate is from weight gain measurements unless noted otherwise. The 1σ uncertainties are also listed.

‡ The rate from ion chromatography analyses of SO_4^{2-} content of reacted sample

§ The rate from scanning electron microscope (SEM) measurements of anhydrite layer thickness and porosity.

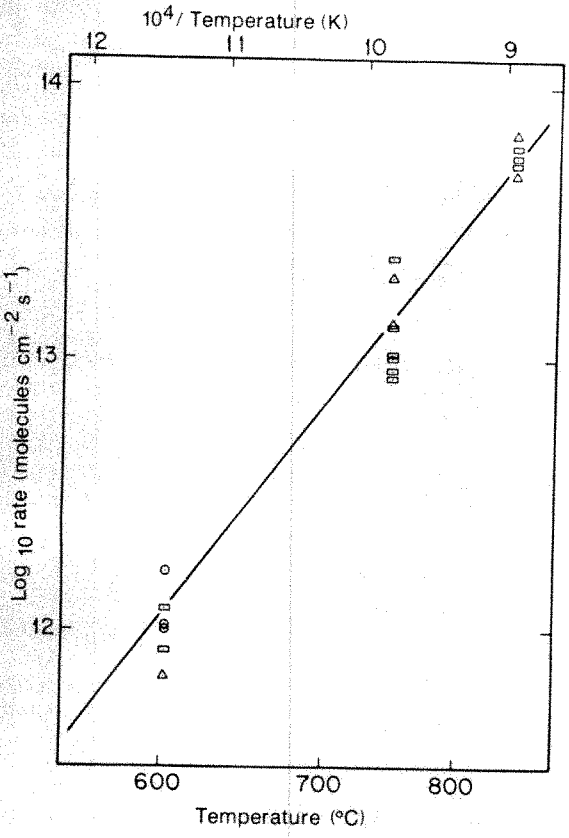


Fig. 2 Arrhenius plot of rate data from Table 1 against $1/T$. The line is a weighted linear least-squares fit, and indicates an activation energy of 126.8 ± 5.4 kJ mol^{-1}. Rates determined from independent methods (weight gain (rectangles), SEM (triangles), SO_4^{2-} analyses (circles)) for the same sample agree within the 1σ experimental uncertainties.

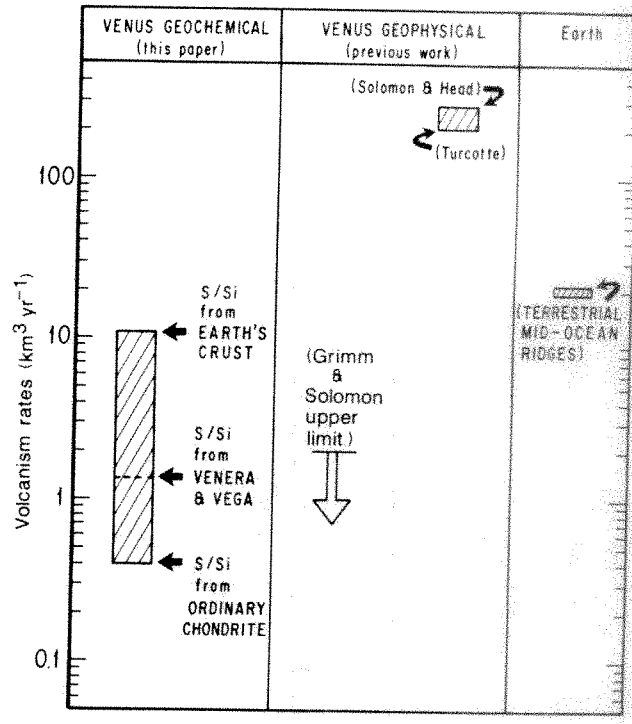


Fig. 3 Geochemical and geophysical estimates of the rate of volcanism on Venus, compared with the terrestrial rate. Three plausible models for the S/Si ratio of erupted material on Venus yield rates of ~0.4 to ~11 km^3 yr^{-1} (see text). Adopting the Venus surface S/Si ratio from Venera 13, 14 and Vega 2 analyses[5,6] yields a rate of ~1 km^3 yr^{-1}, or about 20 times less than the rate of volcanism on Earth. The terrestrial rate of ~20 km^3 yr^{-1} is based on a plate creation rate[27] of ~3 km^2 yr^{-1} and a crustal thickness of ~6 km.

sion of SO_2 into H_2SO_4 cloud particles[21], requires a comparable sulphur source. This source must be endogenic, because infalling cosmic material cannot supply enough sulphur. The calculated SO_2 removal rate on Venus is equivalent to $\sim 2.8 \times 10^{13}$ g sulphur per year. However, the measured terrestrial flux of infalling material, which is roughly applicable to Venus because of similar sizes and masses, is only $\sim 7.8 \times 10^{10}$ g yr^{-1} (ref. 22), or only $\sim 4.6 \times 10^9$ g S yr^{-1} assuming the most sulphur-rich CI chondrite composition[23].

The most plausible endogenic source is volcanism, which has occurred on Venus in the past[24], and which may have led to increased SO_2 levels above the Venus cloud-tops observed by the PV orbiter[25,26]. The rate of volcanism required to balance SO_2 depletion by reactions with calcium minerals on the Venus surface depends on the sulphur content of the erupted material (gas and magma). If this material has an overall sulphur/silicon mass ratio of 0.03 (the weighted mean of the Venera 13, 14 and Vega 2 surface analyses[5,6]), the corresponding rate of volcanism is ~1 km^3 yr^{-1}. Figure 3 illustrates that this rate is ~20 times smaller than the terrestrial value[8,27]. Two other plausible models for the S/Si ratio of the erupted material on Venus are also shown: S/Si ≈ 0.1, as in ordinary chondrites[23], giving a rate of ~0.4 km^3 yr^{-1} and S/Si ≈ 0.004, as in the Earth's crust[28], giving a rate of ~11 km^3 yr^{-1}. All rates will scale as ε^{-1}, where ε is the sulphur degassing efficiency for erupted material ($0 < \varepsilon \leq 1$). However, ε is likely to be close to unity because sulphur gases are expelled directly into the atmosphere, and hot, reactive sulphur-bearing magmas will rapidly lose sulphur to the atmosphere. Our experiments on the chemical weathering by CO_2 of a possible magmatic sulphur-bearing mineral pyrite FeS_2 indicate complete loss of sulphur in hours at sub-magmatic temperatures (~980 °C).

Figure 3 also illustrates that widely discordant volcanism rates have been estimated by two different geophysical methods. Rates of ~200–300 km^3 yr^{-1} are estimated by scaling terrestrial heat production to Venus and transporting the outward heat flux solely by volcanism[8,9], and an upper limit of 2 km^3 yr^{-1} is estimated by analysis of impact-crater areal densities on Venera 15, 16 radar images[7]. The present, geochemically derived volcanism rate is independent of these two methods and can be used, in principal, to test if either (or neither) is correct. In particular, the values computed by the former geophysical method do not agree with our geochemical values. This work also suggests that on average Venus may be less volcanically active than the Earth. Finally, the geochemically estimated volcanism rate can also be used to constrain water loss on Venus through oxidation of FeO-bearing basalt[3]. This work is in progress and will be reported elsewhere.

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Laboratory model of a planetary eastward jet

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The concentration of eastward planetary currents into strong wavy jets is a major feature of the atmospheric general circulation (for example, the jet stream) and of oceanic currents (for example, the Gulf Stream east of Cape Hatteras). These strongly nonlinear jets are, however, not well understood. Here we describe experiments in which we have produced similar jets in a rotating annulus with mechanical forcing: the Coriolis force acting on fluid pumped radially inward from a ring of sources to a ring of sinks results in a strong eastward jet. For a wide range of parameters the jet has a robust wavy shape, as shown in Fig. 1. The wave velocity and wavelength of these Rossby waves are accounted for by simple scaling arguments. Experiments with dye injected into the fluid show that the jet acts as a barrier to tracer transport (see Fig. 1*e*), much as the southern polar night jet may act as a barrier to the transport of ozone from lower latitudes into the polar region[1]. Our observations are insensitive to the details of the arrangement of the sinks and sources.

The annular tank (86.4 cm in diameter) is rotated rapidly (up to 4 Hz) to achieve an essentially two-dimensional geostrophic motion, as in large-scale planetary flows. (In a geostrophic flow the Coriolis force is balanced by pressure gradients[2].) The two-dimensionality of our flow is evident in photographs such as Fig. 1*a*, in which particles at different heights in the fluid are seen to produce parallel streaks. The rigidly rotating annulus has an inner radius $r_1 = 10.8$ cm, an outer radius $r_2 = 4r_1$, and a depth that increases linearly with radius from 17.1 cm at r_1 to 20.3 cm at r_2. The conical bottom mimics the beta effect in a planetary atmosphere (the variation of the Coriolis force with latitude). The upper surface of the fluid (water) is in contact with a flat cover. In most of the experiments, including those shown in Fig. 1*a*, *b* and *e*, an eastward jet was produced by pumping fluid continuously from six sources in the bottom of the tank at $r = 35.1$ cm into six sinks at $r = 27.0$ cm; each source and sink consists of 400 holes (0.8 mm in diameter) within a 3.8-cm-diameter circle (these circles are inside the dark disks in Fig. 1*b* and *e*).

For low total pumping rates $F < 20\ \mathrm{cm^3\ s^{-1}}$, each source or sink produces a vortex that stays in the neighbourhood of a port, with sinks producing cyclonic vortices and sources producing anticyclonic vortices. For larger pumping rates, however, the vortices are advected eastward, resulting in a continuous jet. The strength of the jet is controlled by a balance of the total torque arising from the Coriolis force on the radially pumped fluid with the total torque arising from the friction force. The friction arises primarily from the Ekman boundary layers on the top and bottom surfaces of the tank; the tank was made deep to minimize the relative effect of this dissipation. The characteristic time for the dynamics, an oscillation period of the jet, is typically an order of magnitude shorter than the Ekman dissipation time $\tau = h_0/2(\Omega\nu)^{1/2}$ (where h_0 is the mean depth of the fluid, Ω is the angular velocity and ν is the kinematic viscosity); in other words, the Rossby number is typically 0.1. Because the dissipation is weak and the Coriolis force is large, the azimuthal velocity of the jet is typically two orders of magnitude greater than the average radial velocity, and the behaviour of the jet is determined mainly by inertial effects.

The waves on the jet are not attached to the sources and sinks—the crests move eastward with a speed of about 15% of the maximum velocity of the jet. The number of waves varies from 3 to 8, depending on Ω and F (the ranges studied are $6 < \Omega < 25\ \mathrm{rad\ s^{-1}}$ and $15 < F < 370\ \mathrm{cm^3\ s^{-1}}$). For a given Ω and F, the number of waves is reproducible but the wave amplitude and wavelength fluctuate in time. The quasi-steady wave pattern is the result of a balance of westward propagation of Rossby waves and eastward advection.

Quasi-geostrophic flows can be described in terms of the potential vorticity, $q(r, \theta)$, which is given in this situation (that is, uniform fluid density and a topography with a small constant slope) by[2]

$$q = \omega + \beta r \tag{1}$$

where $\omega = (\nabla \times \mathbf{u})_z$ is the vorticity of a fluid parcel relative to the rotating tank, $\beta = 2\Omega s/h_0$ is the coefficient of the beta effect ($s = -0.1$ is the slope of the bottom of the tank), r is the radial coordinate and $\mathbf{u}$ is the fluid velocity. In the absence of viscosity and forcing, q is conserved for each fluid parcel, that is,

$$Dq/Dt \equiv [\partial/\partial t + (\mathbf{u}\cdot\nabla)]q = 0 \tag{2}$$

In our experiment the potential vorticity is nearly conserved—the weak dissipation is balanced on average by the forcing.

The velocity field in the annulus is determined from measurements of the length of particle streaks in time-exposure photographs, such as that in Fig. 1*a*. The velocity vectors from typically 300 particle streaks are fitted with cubic splines[3], and the resultant velocity field is differentiated to determine the potential vorticity at any point in the fluid. We find that there is a steep gradient of potential vorticity in the middle of the jet, as Fig. 1*c* and *d* illustrates, whereas away from the centre of the jet the potential vorticity is fairly uniform. (A gradient in potential vorticity is necessary for the propagation of Rossby waves.) Juckes and McIntyre[4] observed a similar steepening of an initially smooth gradient in their numerical study of a single-layer model of a terrestrial stratospheric polar vortex. At low latitudes the initially axisymmetric flow around the vortex was unstable and rapidly became turbulent, creating a well mixed state, whereas the polar vortex remained an essentially isolated structure. Mixing between these two regions took the form of long thin 'tongues' of potential vorticity and was associated with stratospheric wave-breaking[4–6]. This local steepening of potential vorticity gradients seems to be common behaviour for strongly stirred inertial quasi-geostrophic layers and is similar to the steepening of density gradients observed in strongly stirred stratified non-rotating fluids[7].

In our experiment, injected dye is focused into the jet and renders it visible even when the dye is injected at a radius only occasionally reached by the wave crests or troughs. In a two-dimensional non-divergent flow this convergence must be associ-

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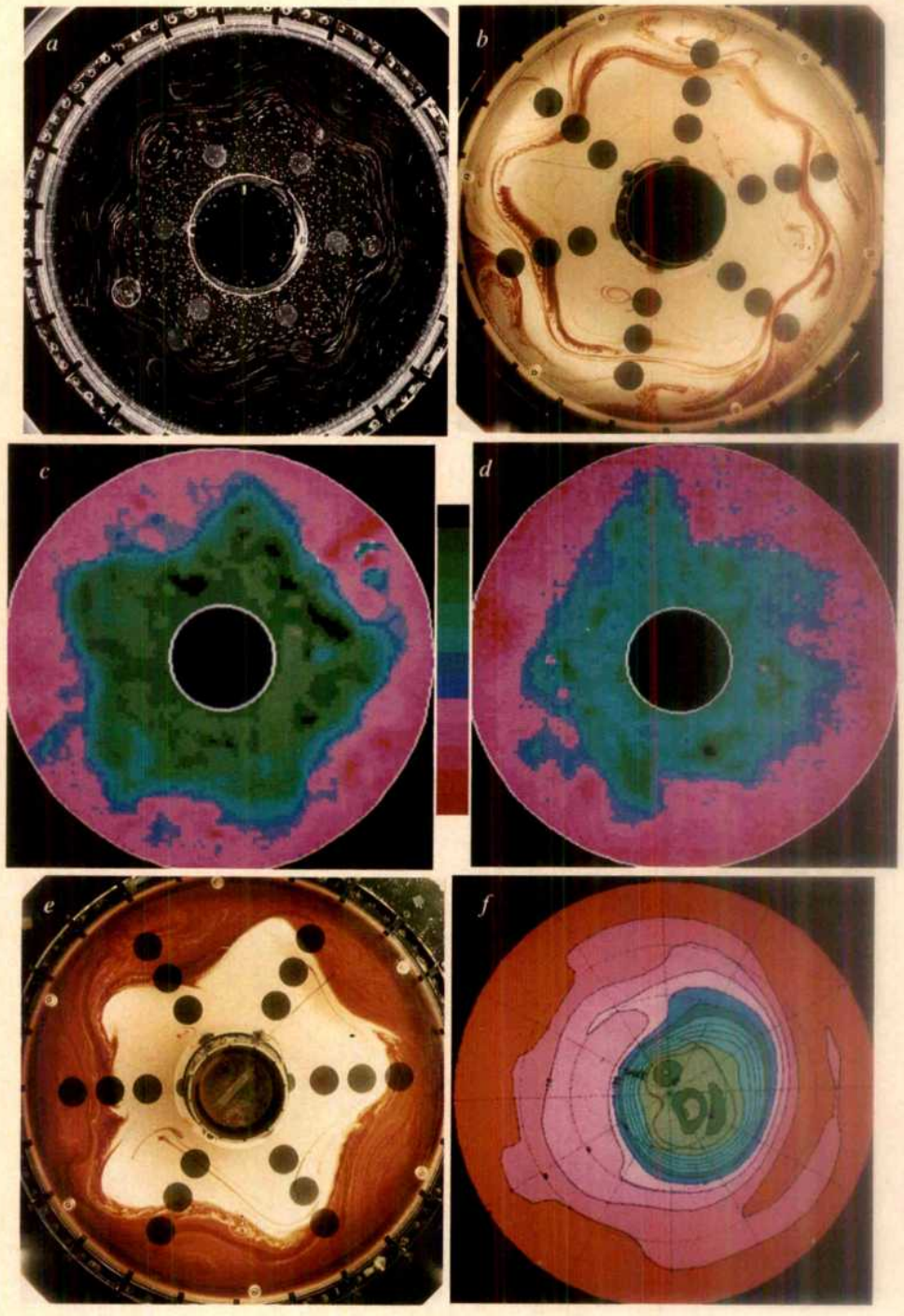


Fig. 1 *a*, A jet with eight waves, visualized with particle streaks photographed in a frame of reference rotating with the tank ($\Omega = 25.1$ rad s^{-1}, $F = 30$ cm^3 s^{-1}; exposure time = 0.5 s). *b*, A jet with seven waves, visualized by continuous injection of dye through a small tube ($\Omega = 18.8$ rad s^{-1}, $F = 40$ cm^3 s^{-1}). *c*, Potential vorticity q for a jet with five waves. The potential vorticity, deduced from particle streak measurements, ranges from -8.1 rad s^{-1} (red) to -0.6 rad s^{-1} (dark green) ($\Omega = 18.8$ rad s^{-1}, $F = 90$ cm^3 s^{-1}). *d*, Potential vorticity q for a jet that formed spontaneously in a slowly decelerating tank with the sources and sinks alternating in a ring at $r = 27.0$ cm. The potential vorticity ranges from -15.0 rad s^{-1} (red) to 2.3 rad s^{-1} (dark green) ($\Omega = 25.1$ rad s^{-1}, decreasing at a rate of 0.012 rad s^{-2}; $F = 137$ cm^3 s^{-1}). *e*, A jet with five waves, photographed more than 500 revolutions of the tank after dye was injected outside the jet ($\Omega = 18.8$ rad s^{-1}, $F = 90$ cm^3 s^{-1}). In *a*, *b* and *e* the jet was produced by pumping fluid from an outer ring of sources to an inner ring of sinks. *f*, Ertel's potential vorticity on an isentropic surface in the upper stratosphere on a calm winter day in the Southern Hemisphere (from ref. 27); contour lines at intervals of 5×10^{-8} Pa^{-1}s^{-1} are distinguished by colour. (The units are those of potential vorticity appropriate to the atmosphere.) A strong potential vorticity gradient in the central region of the jet isolates the polar vortex from the surrounding atmosphere.

ated with an increase of the jet path length, resulting in an increase of the wave amplitude which cannot persist in a steady regime. The growth is limited by a random localized ejection of fluid from the jet which can be observed in the motion of particles suspended in the flow. A particle typically stays inside the jet for several revolutions and is then advected far outward at a wave crest by one of the weak recirculating eddies surrounding the jet. Particles are also advected inwards at wave troughs, but less often. The ejection can be seen in Fig. 1*b* and *e* as tongues of dye. This process slowly fills the space outside the jet with a uniform concentration of dye. When dye is injected far from the jet, it is spread uniformly in the region delimited by the jet, but there is remarkably little transport across the jet for very long periods of time, as Fig. 1*e* illustrates.

These diffusion properties elucidate the dynamics of the potential vorticity, which is advected like a dye; compare the photograph in Fig. 1*e* of a five-wave jet visualized with dye with the plot in Fig. 1*c* of the potential vorticity determined for a five-wave jet. The flow toward the centre of the jet brings the positive potential vorticity produced by the sinks close to the negative potential vorticity produced by the sources, thus inducing a steep gradient. The local ejection of potential vorticity associated with the ejection of fluid particles into the surrounding regions helps to mix potential vorticity and contributes to the small-scale structure seen in Fig. 1*e*. In regions of quasi-uniform potential vorticity the dye spreads quickly and evenly but does not cross the strong potential vorticity gradient in the middle of the jet. The zones of uniform potential vorticity, which cannot support Rossby waves, confine the waves by a nonlinear reflection effect[8,9]. The ejection of particles at wave crests is probably related to the filamentation of potential vorticity observed by Juckes and McIntyre[4], as mentioned above. The

filamentation process has been also observed recently by Dritschel[10] in near-inviscid numerical computations for a circular blob of uniform vorticity surrounded by irrotational fluid. He found that a small perturbation on an initially symmetric vortex creates an irregular region of ever-increasing complexity around the vortex.

Our results are insensitive to the details of the forcing geometry, provided that the flow is eastward with respect to the forcing ports. For example, closing all of the ports on one side of the tank makes virtually no difference to the behaviour of the flow if the total flux F is the same—dye injected away from the centre of the jet is still inhibited from crossing the jet, even in the unforced part of the annulus. Also, a narrow coherent wavy jet is still observed when the distance between the sources and sinks is doubled. A similar, although somewhat less regular, structure is obtained even using a single ring of alternating sources and sinks together with a mean eastward flow produced by a slow deceleration of the tank; see Fig. 1*d*. Seen in a different frame of reference, this situation is equivalent to a forcing that propagates westward in a fluid at rest; this forcing has been used by others[11–13] to demonstrate the production of a mean zonal flow by eddies[14]. In our experiment, in which the effect of friction is less important than in previous experiments, the propagating forcing leads to an inertial eastward jet.

The total angular momentum of the jet results from a balance of the total torque introduced by the forcing with the total torque caused by friction in the boundary layers. This balance leads to the relation $UL = (\Omega \pi d / \pi \langle r \rangle h_0) F$, where the distance d between the rings of sources and sinks is assumed to be small compared with the mean radius $\langle r \rangle$ of these two rings. Here U is a velocity scale and L is the characteristic jet width. Considering the balance between advection and the beta effect yields the classic condition $U/L^2 \approx \beta$. The two relations involving U and L lead to[15]

$$U = a(sd^2/2\pi^2 \nu h_0 \langle r \rangle^2)^{1/3} (F\Omega)^{2/3} \quad (3)$$

$$L = b(h_0 d / 4\pi s \langle r \rangle \nu^{1/2})^{1/3} \Omega^{-1/6} F^{1/3} \quad (4)$$

where a and b are non-dimensional parameters that in general depend on the velocity profile and the exact definitions of U and L. The observed azimuthally averaged velocity, $U(r) = \langle u_\theta(r, \theta) \rangle_\theta$, fits a $\mathrm{sech}^2(r/L)$ profile fairly well for the full range of experimental conditions examined. Moreover, the data are described reasonably well by the scaling relations (3) and (4) with a and b given by the values for a sech^2 profile, $a = (3/8)^{1/3}$ and $b = (1/3)^{1/3}$, as Fig. 2*a* shows for the velocity data. Thus the jet has the same basic structure over a wide range of parameters.

Some qualitative aspects of the jet can be understood using linear theory. Because the perturbations are large, the best form for modelling the zonal flow is not obvious; for simplicity we take the azimuthally averaged flow. The measured profile satisfies the Rayleigh–Kuo criterion of marginal stability[16], which states that the maximum of $\mathrm{d}^2\langle U(y) \rangle / \mathrm{d}y^2$ is equal to $|\beta|$ (where the jet curvature is neglected and y represents the radial direction). When the forcing is turned on, the initially unstable jet profile is broadened by the growth of instabilities until it is stabilized by the beta effect into a marginally stable shape. There are two predicted modes of linear instability, varicose and sinuous, but only the latter is unstable for an eastward jet satisfying the Rayleigh–Kuo marginal stability condition[17]; this explains our observation of a wavy shape. The wavenumber k for the instability is $2^{1/2}L^{-1}$ for a jet with a sech^2 profile. The observed number of waves is in qualitative but not quantitative agreement with this linear theory, as Fig. 2*b* shows. The departure from linear theory is not surprising as the experiments are in a nonlinear regime involving finite amplitude waves and wave-breaking.

Our flow is barotropic (the density is uniform and the velocity field does not vary with height), whereas the atmosphere and oceans are baroclinic (the density and velocity depend on altitude), although planetary-scale flows in the middle stratosphere often exhibit deep vertical structure similar to barotropic flows. Some elegant laboratory studies of Rossby waves have been conducted previously on a baroclinic system, a heated rigidly rotating annulus of water[18–22]. Wavy coherent jets were obtained over a wide range of parameters, but their three-dimensional baroclinic structure is difficult to analyse. We chose to study a barotropic system because it is simpler, yet it exhibits many of the phenomena exhibited by baroclinic flows, including, in particular, Rossby waves. The observation that phenomena found in barotropic flows also occur in baroclinic flows has been termed the 'principle of barotropic analogy' by McWilliams[23].

The behaviour of the potential vorticity in our experiments is analogous to the behaviour of Ertel's potential vorticity in the atmosphere on an isentropic surface[24]. Above the lower troposphere in the Earth's atmosphere, where most of the diabatic and friction effects occur, the flow is dominated by advective effects and Rossby wave propagation; gradients of the potential vorticity on an isentropic surface are due to the tilt of the constant-density surfaces and the curvature of the Earth. Rossby waves propagating westward in an upper atmospheric layer or in the ocean are often amplified by the baroclinic instability[24], just as a steady Rossby wave is amplified by the forcing in our tank. The concentration of the potential vorticity gradient in our jet surrounded by zones of uniform potential

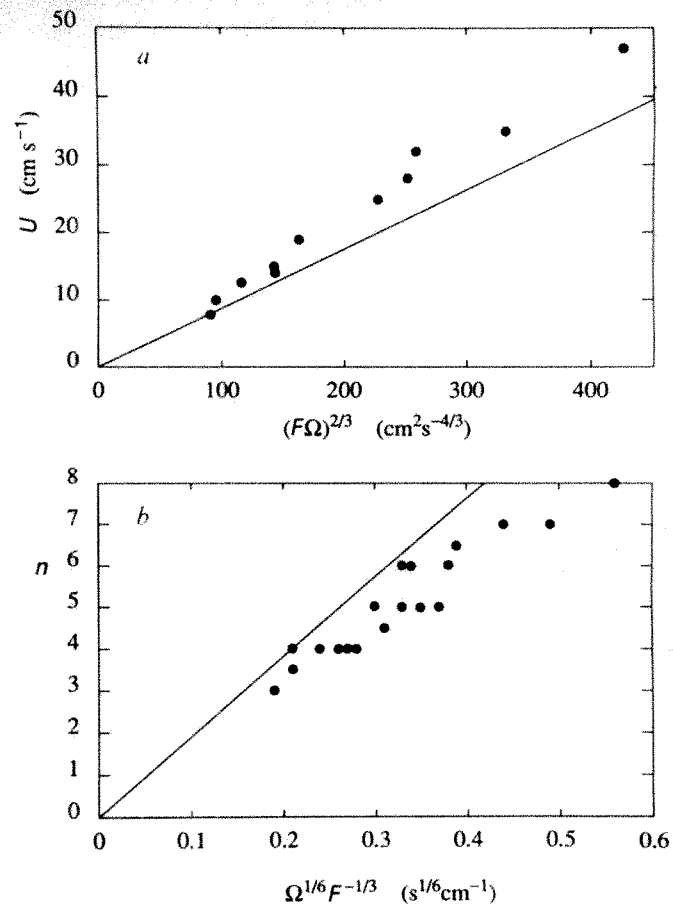


Fig. 2 *a*, The maximum of the azimuthally averaged velocity of the jet, U, compared with the prediction of a marginal stability analysis (diagonal line). *b*, The observed number of waves compared with the value predicted by linear theory (solid line), $n = k\langle r \rangle = 2^{1/2}L^{-1}\langle r \rangle$. In the neighbourhood of a transition the number of waves fluctuates between n and $n+1$; these points are plotted as half-integer values of n.

vorticity is similar to that observed in the atmosphere and the ocean[25,26]; see, for example, Fig. 1*f*. Filamentary mixing across a potential vorticity gradient, such as that illustrated by Fig. 1*e* and *f*, may also play an important role in mixing of atmospheric tracer elements such as ozone[1,27,28].

In conclusion, we have described a relatively simple system for studies of instabilities and turbulence in barotropic jets at high Reynolds number ($\sim 10^5$). Our experiments suggest that jets in planetary scale flows can be inertial structures that are largely independent of the details of the forcing, depending mainly on the total energy and momentum. We find that the wavy eastward jets serve as a striking barrier to particle transport. The potential vorticity barrier has also been found to be remarkably complete in numerical experiments[4]. The laboratory and numerical studies and an analysis of data on the Antarctic atmosphere have led to the vortex-isolation hypothesis of McIntyre[29]: "in the real Antarctic lower stratosphere, the sealing-off of the vortex core from its surroundings might be practically complete".

The narrow wavy structure found for eastward jets in our experiments contrasts markedly with the turbulent flow containing persistent vortices found for westward jets in earlier experiments in the same apparatus[15]. The relevance of these observations to planetary-scale flows should be examined further in future studies.

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Geochemical evidence for suppression of pelagic marine productivity at the Cretaceous/Tertiary boundary

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The normal, biologically productive ocean is characterized by a gradient of the $^{13}C/^{12}C$ ratio from surface to deep waters. Here we present stable isotope data from planktonic and benthic microfossils across the Cretaceous/Tertiary boundary in the North pacific, which reveal a rapid and complete breakdown in this biologically mediated gradient. The fluxes of barium (a proxy for organic carbon) and $CaCO_3$ also decrease significantly at the time of the major marine plankton extinctions. The implied substantial reduction in oceanic primary productivity persisted for ~0.5 Myr before the carbon isotope gradient was gradually re-established. In addition, the stable isotope and preservational data indicate that environmental change, including cooling, began at least 200 kyr before the Cretaceous/Tertiary boundary, and a peak warming of ~3 °C occurred 600 kyr after the boundary event.

The section at DSDP Site 577 on the Shatsky Rise (32°26′ N, 157°43′ E) was collected by hydraulic piston core and yielded the first continuous, undisturbed sequence of pure nannofossil ooze (>90% $CaCO_3$) across the Cretaceous/Tertiary (K/T) boundary. Because of the relatively shallow burial depth of the K/T boundary sequence (<110 m below the sea floor (m.b.s.f.)[1]), the calcareous microfossils from Site 577 have not undergone significant recrystallization related to burial[2–4]. The position of the K/T boundary is placed at the last appearance of planktonic foraminifera of the *Abathomphalus mayaroensis* zone[4] and first appearance of calcareous nannofossils of the *Marcalius inversus* zone[2], which also coincides with peak abundance of magnetite-glauconite spherules at 109.09 m.b.s.f. (refs 4, 5). A peak irridium anomaly of 61 ng cm^{-2} also occurs at the boundary, as defined by nannofossils, in adjacent Hole 577B (ref. 6). The palaeodepth of Site 577 is estimated as having been 2,400 m at the time of the K/T boundary[3]. The record at DSDP Site 577 is adequate to resolve the nature of environmental changes in pelagic settings that occurred in conjunction with the K/T boundary event, and provides important constraints on hypotheses for extinction.

To reconstruct changes in the surface-to-deep-water carbon isotope gradient of total dissolved carbon (TDC), stable-isotope analyses were conducted on fine fraction (<63-μm) carbonate and several species of planktonic and benthic foraminifera in closely spaced samples over a 10-m interval spanning the K/T boundary[3,4,7] (Fig. 1). The $\delta^{13}C$ of fine-fraction carbonate (primarily calcareous nannoplankton) and of planktonic foraminifera from this site decreases by about 1.5‰ at the K/T boundary relative to values just below the boundary. The $\delta^{13}C$ values of biserial benthic foraminifera *Aragonia* and of trochospiral benthic foraminifera *Nuttallides*, *Gavelinella* and *Gyroidinoides* show an enrichment across the boundary converging with $\delta^{13}C$ values of both planktonic foraminifera and fine-fraction carbonate just above the boundary (Fig. 2).

Convergence of $\delta^{13}C$ values of planktonic foraminifera, nannoplankton (fine-fraction) and benthic foraminifera indicates that the biologically mediated gradient in carbon isotopes between the surface and deep waters, which is characteristic of productive oceans, effectively disappeared at the K/T boundary. Such conditions have been termed a 'Strangelove' ocean on the basis of predictions for events following a mass extinction[8,9]. The loss of a surface-to-deep-water carbon isotope gradient could only be caused by a major reduction in primary production or by extremely rapid rates of oceanic turnover. At Site 577 the lack of a carbon isotope gradient suggests that a poorly productive ocean persisted for at least 0.5×10^6 yr following the K/T boundary event (Fig. 2). The gradual development of a surface-to-deep-water $\delta^{13}C$ gradient in the period following this interval probably represents a progressive increase in primary production.

The changing offset of $\delta^{13}C$ between biserial and trochospiral

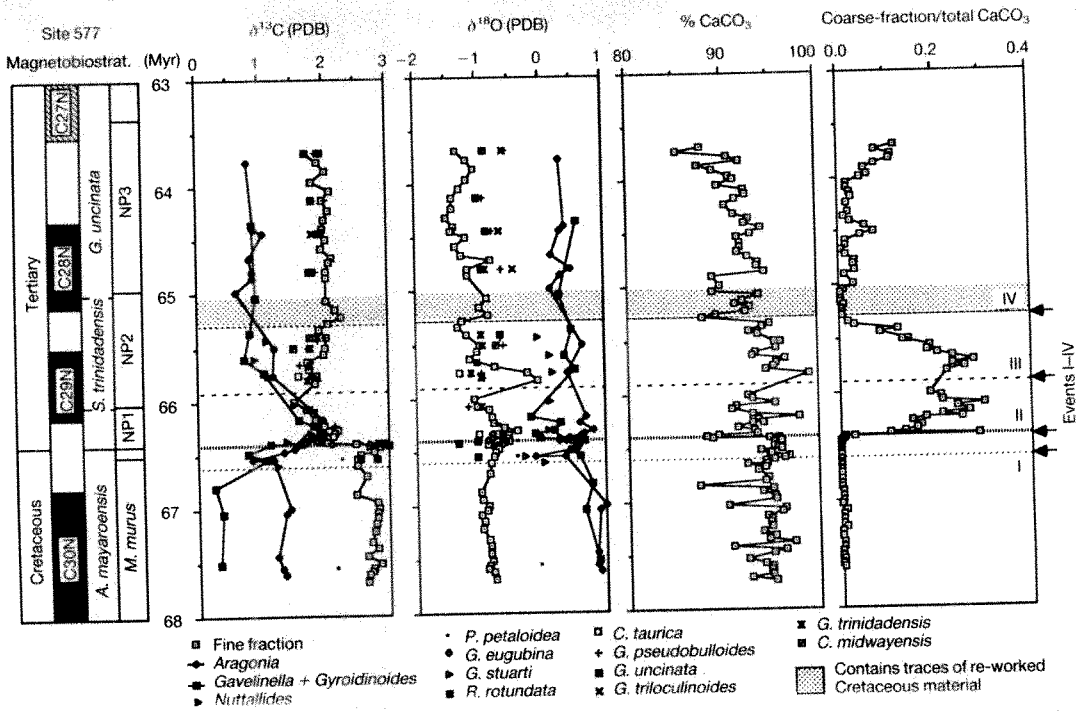


Fig. 1 Age plots of carbon and oxygen stable isotope compositions of fine-fraction carbonate (<63 μm), benthic foraminifer *Aragonia*, trochospiral genera *Gavelinella* + *Gyroidinoides*, and *Nuttallides*, various species of planktonic foraminifera, and carbonate content of whole rock samples. All stable isotope data are expressed in the delta notation, δ, in ‰ relative to the PDB marine carbonate standard[27]. The final column shows the weight ratio (%) of coarse fraction (>63 μm) to total carbonate. Age determinations were made using available magneto-stratigraphy[28] and nannofossil stratigraphy[13] (presented on the far left) and the most recently published timescale[29]. Four separate events are recognized in the evolution of the stable isotope and carbonate content records and are denoted by the four dashed lines. The shaded area represents a portion of the sequence which contains re-worked Cretaceous fossils. The gap in sampling from 65.8 to 65.95 Myr encompasses the interval of an organic geochemistry core removed on board ship.

benthic foraminiferal genera (Fig. 1) is particularly interesting because of the observation that many species of modern benthic foraminifera do not reside directly on the sea floor but instead within the upper few centimetres of sediment, and therefore secrete their tests in carbon isotope equilibrium with the surrounding pore waters rather than with bottom waters[10]. *In situ* decay of organic matter in the upper few centimetres of sediment produces a pore-water $\delta^{13}C$ gradient with a magnitude which is, in part, a function of the accumulation rate of organic carbon (C_{org})[11]. Convergence of $\delta^{13}C$ values of *Aragonia* and that of *Gavelinella* + *Gyroidinoides* across the K/T boundary may indicate that the pore-water $\delta^{13}C$ gradient is eliminated as a result of the reduction in one C_{org} flux to sediments. The absence of benthic foraminiferal carbon isotope differences during the recovery period suggests that new C_{org} flux levels were lower than that of the latest Cretaceous.

Other evidence which suggest earliest Palaeocene reduced primary productivity involves carbonate and barium mass-accumulation-rate data. In the absence of changes in the rate of dissolution, the rate of accumulation of biogenic carbonate should be proportional to production in surface water. Carbonate accumulation rates decline on average by a factor of four across the K/T boundary in all pelagic marine sequences examined[3]. In general, preservation of calcareous microfossils improves across the K/T boundary at most localities[4,12]. Therefore, the decrease in carbonate accumulation rates from Maestrichtian to the early Palaeocene cannot be easily attributed to increased rates of dissolution and must have resulted from a decrease in the rate of $CaCO_3$ production in surface waters.

The distribution of dissolved barium in the modern oceans is nutrient-like and appears to be mediated by biological activity[13,14]. In the modern Pacific Ocean, Ba and organic-carbon sediment-trap fluxes are commonly proportional[15]. Because sediment pore waters are near saturation with respect to barite[16], barite is typically preserved in sediments whereas C_{org} is oxidized. Thus, Ba accumulation rates can be used as a proxy for C_{org} flux to the sediments[17]. At Site 577, Ba accumulation rates decrease from >0.2 to <0.1 mg cm^{-2} per 10^3 yr across the K/T boundary (Fig. 3), reflecting the decreased rain rate of C_{org}.

We have recognized four discrete episodes in the evolution of the stable-isotope and carbonate records across the K/T boundary and in the early Palaeocene at Site 577. The first of these events begins at least 10^5 yr before the main plankton extinctions and the last occurs nearly 1.5×10^6 yr later. We believe that these events represent significant changes in oceanic fertility, circulation and climate, which are accompanied by consequent effects on biotic evolution.

Event I (66.6 Myr), which precedes the K/T boundary event, is marked by enrichment in both planktonic and benthic microfossil carbon isotope records, accompanied by a slight increase in carbonate content and an improvement in microfossil preservation. In addition, Ba accumulation rates decrease. The increase in $\delta^{13}C$ of both the planktonic and benthic records probably indicates a change in $\delta^{13}C$ of the oceanic TDC reservoir. This could have resulted from increased organic-carbon burial on continental margins (or decreased carbon fluxes from weathering) that are related to the latest Cretaceous transgression, which began at about 66.7 Myr (ref. 18). In addition, there may have been changes in deep-water sources, increased oxygenation of deep waters and/or lower organic-carbon flux at Site 577 at this time. Enriched fine-fraction $\delta^{18}O$ values also indicate cooling of surface waters; this suggests that the rate of oceanic turnover and ventilation of the deep reservoir increased before the K/T boundary as the result of global cooling and intensification of thermohaline-driven circulation. The increase in relative proportions of the temperate planktonic foraminiferal

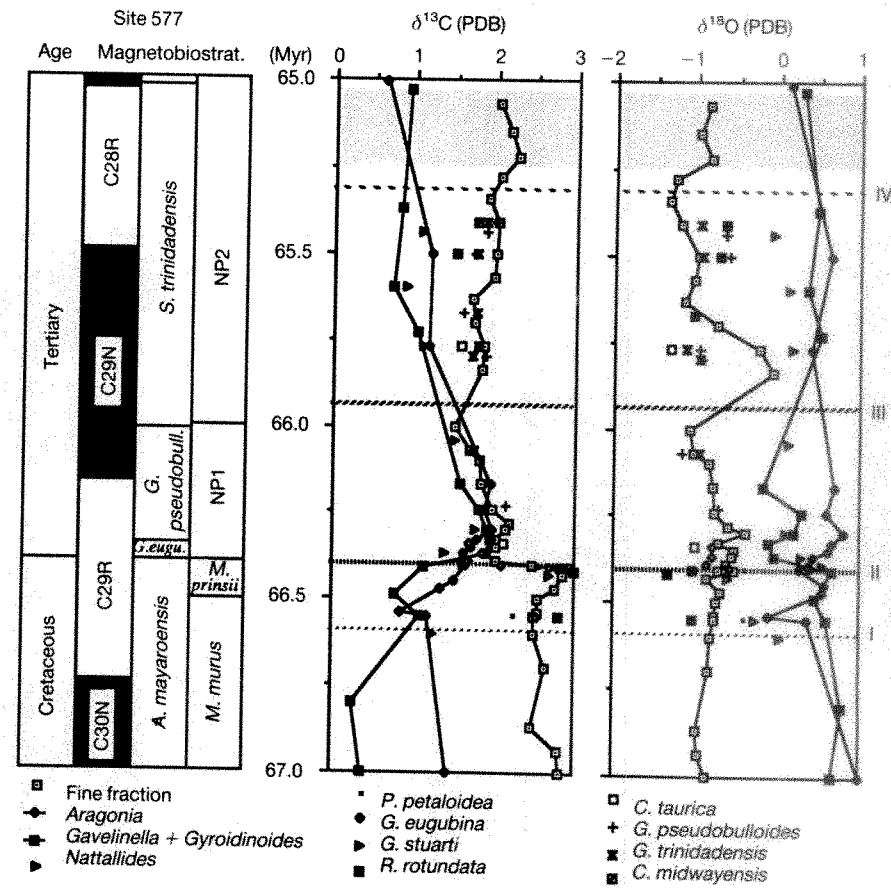


Fig. 2 Expanded 2-Myr record across the K/T boundary of the stable carbon and oxygen isotope data from Fig. 1. Shaded area represents a zone containing traces of re-worked Cretaceous microfossils.

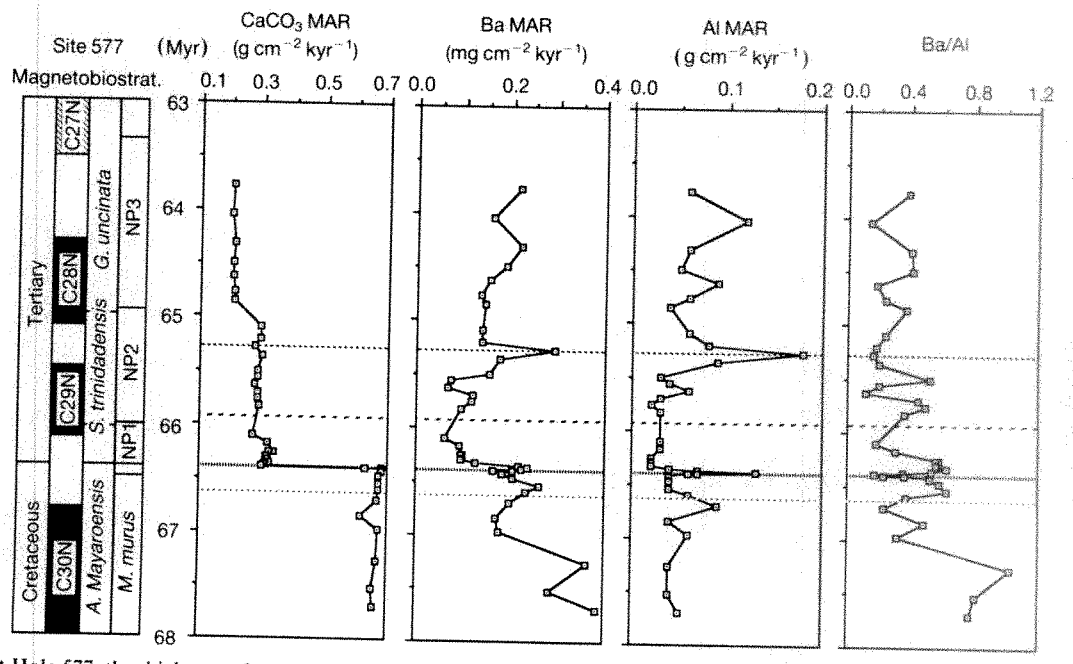


Fig. 3 Age plots of barium (Ba), aluminium (Al) and carbonate mass accumulation rates (MAR), and Ba/Al ratios, at Site 577. The accumulation rates were calculated using physical properties data from the DSDP Leg 86 volume and sedimentation rates based on the magneto- and nannofossil stratigraphy at left. Age assignments for the immediate post-boundary interval were averaged from the top of the Cretaceous (109.1 m.b.s.f., 66.4 Myr) to the base of Chron 29N (108.4 m.b.s.f., 66.17 Myr). As a result, we cannot resolve changes in accumulation rates that occurred on timescales of 10^4 yr or less at Site 577. Because the Base of Chron 30N (C30N) or the *M. murus* zone were not reached at Hole 577, the thickness of the *M. murus* zone (8.48 m) was extrapolated from adjacent Hole 577A. The Ba/Al ratio represents weight ratio of Ba to Al_2O_3.

genus *Heterohelicidae* at this low latitude provides additional support for cooling before the K/T boundary event[4].

Event II: K/T boundary (66.4 Myr), the main plankton extinction and productivity crisis, features the rapid collapse of the surface-to-deep-water carbon isotope gradient and decreases in carbonate and Ba accumulation rates described above. A puzzling aspect of this event is the 100-fold increase recorded in the concentration of foraminifera relative to total carbonate (Fig. 1). There are three possible explanations for this phenomenon: (1) intensified deep circulation resulting in winnowing of the fine fraction; (2) less fragmentation and improved preservation of the more dissolution-susceptible planktonic foraminifera as a result of deepening of the carbonate compensation depth (CCD) and/or lowered rates of *in situ* dissolution brought about by decreased C_{org} decay within sediment pore waters; and (3) an ecological trophic-structure response to the

extinctions and environmental change in which calcareous nannoplankton are replaced by siliceous or organic-walled phytoplankton. We favour preservation changes (2) as the possible origin of the increase in foraminifera concentrations. It is generally accepted that coccoliths tend to be more dissolution-resistant than foraminifera and that calcite dissolution above the calcite saturation horizon is driven mainly by titration by metabolic CO_2 derived from C_{org} decay at or near the sediment–water interface[19]. Dissolution at the shallow palaeodepth of the Shatsky Rise near site 577 would have been controlled to a large extent by metabolic CO_2 production. Decay of C_{org} at the sediment–water interface would decline as a result of reduced C_{org} flux during the 'Strangelove' ocean event making bottom and pore water less corrosive to calcite. Furthermore, taking into account the large initial decrease in $CaCO_3$ accumulation that occurred as a result of the productivity crisis, and assuming that riverine input of inorganic C and alkalinity remained constant, one would expect a rapid deepening of the CCD and much improved preservation of foraminifera across the K/T boundary. Thus we explain the increased foraminiferal flux in the Palaeocene, implied by the higher relative proportion of foraminifers, by enhanced preservation.

Event III (65.9 Myr) is characterized by the gradual return of the surface-to-deep-water carbon isotope gradient, a slight increase in Ba accumulation rates and a gradual decrease in the planktonic foraminifera concentrations. These changes indicate that surface-water productivity began to increase ~0.5 Myr after the K/T boundary event, albeit at relatively low levels. Climate fluctuations of several degrees are indicated by variations in nannofossil and foraminiferal $\delta^{18}O$. Rates of evolution of planktonic forams remained high[20], suggesting continuing environmental instability.

Event IV (65.3 Myr) signals the full expression of the surface-to-deep-water carbon isotope gradient and a decrease in planktonic foraminiferal concentrations to normal values. Aluminium accumulation rates also increased at this time. Because Al accumulation rates are probably a reflection of flux of aeolian detritus to this site[21], the increased Al accumulation rates may represent climate change at this time and possibly an increase in wind strength. Environmental stability is indicated by a nearly constant $\delta^{13}C$ gradient, relatively stable $\delta^{18}O$ values and low rates of change of planktonic foraminiferal faunas in the *G. uncinata* zone[20].

The extinction of phyto- and zooplankton and disappearance of the carbon isotope gradient is abrupt and occurs over a period of less than 10^4 yr. It is unlikely that a gradual environmental change occurring over a longer period of time (that is, 10^5–10^6 yr) could have resulted in such a rapid and extreme collapse of the marine ecosystem unless some as yet unknown environmental threshold was exceeded[22]. The instantaneous environmental stress that would be brought about by a bolide impact provides a possible explanation for the collapse of primary productivity and its coincidence with an abundance of spherules and platinum-group metals at the K/T boundary, as exhibited at Site 577. It fails to explain, however, why primary productivity remained low for such a long period after the event.

It is possible that the 0.5-Myr 'Strangelove' ocean may represent the normal recovery time for the biosphere following a sudden mass extinction event. An alternative explanation is that the slow recovery of primary productivity was the result of climate and oceanic circulation instability. The rate of speciation was extremely high for the first several 10^6 years above the K/T boundary at Site 577, with one assemblage of planktonic foraminifera quickly replacing another[20]. Because the distributions of planktonic foraminifera are limited to particular water masses, the high turnover rate of assemblages suggests that surface-water conditions changed rapidly during the Danian. As a consequence, an efficient, highly structured ecosystem may have been incapable of developing. The origin of this climatic instability is uncertain but may prove to be related to feedback mechanisms involving the global carbon cycle[9,23–25]. Models predict that, following a rapid extinction event, atmospheric p_{CO_2} would have increased by as much as a factor of two or three on a timescale of several oceanic mixing cycles[26], as the result of nearly complete cessation of oceanic productivity. Such increases in p_{CO_2} would be expected to result in global warming. The nannofossil data, which represent the most complete time series, suggest a gradual 3 °C warming of surface waters during the initial 0.5 Myr of the Palaeocene.

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A brachiopod calcite record of the oceanic carbon and oxygen isotope shifts at the Permian/Triassic transition

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The Permian/Triassic (P/T) mass extinction was the most dramatic of all such events but its cause has thus far remained elusive[1–3]. Carbon and oxygen isotope analyses may offer clues to this puzzle because their results identify major changes in the Earth's exosystem[4,5]. Several studies have already dealt with carbon and oxygen isotope records at the P/T transition[2,6–11], but we present here empirical data derived from the brachiopod shell calcite, whose isotope composition generally corresponds to that of the sea water in which the animals lived originally[12–15]. These data allow us to assess quantitatively the magnitude of changes in the Earth's exosystem during that critical time interval.

We sampled the Upper Permian Kapp Starostin Formation[16]

at a number of localities in West Spitsbergen (Fig. 1). This formation consists mainly of cherts and carbonates associated with fine-grained terrigenous deposits, which accumulated in open marine environments of a broad epicontinental sea[17]. The conodonts found in the section at Kapp Starostin demonstrate the latest Leonardian age of the basal part of the formation and strongly suggest a Changsinghian (latest Permian) age for the upper part of the section[18]. In central West Spitsbergen, deposits of the Kapp Starostin Formation pass, without any evidence for discontinuity, into the overlying marly shales of the Vardebukta Formation[19], which contain, in this area, Griesbachian (early Triassic) conodonts[20] and molluscs[21] and, higher up, Dienerian ammonoids[22]. Thus the area appears to represent one of the few continuous stratigraphic records across the P/T transition. Time correlation among individual sections within the Kapp Starostin Formation (Fig. 2) has been achieved by means of event stratigraphy[23].

The samples we analysed for carbon and oxygen isotopes are brachiopod shells with fully preserved primary microstructure and without any evidence for allochthoneity. To ensure consistency of the isotope analysis, we focused on a single brachiopod taxon, the Productacea (although we also took some other fossil and rock samples for comparative purposes). This material is particularly suitable for such study because brachiopods fractionate only insignificantly the carbon and oxygen isotopes they take up from the sea water for shell development, and the isotope composition of their low-magnesium calcite is generally not affected by the diagenetic alterations that so often obscure the chemistry of rock matrix and skeletal calcite[12-15,25-27].

The carbon and oxygen isotope composition of our samples was determined by mass spectrometry on CO_2, obtained by calcite reaction with 100% H_3PO_4; the standard error of single measurements is less than 0.08‰ (ref. 28). The results are expressed here in $\delta^{13}C$ and $\delta^{18}O$ notation (relative to the PDB standard, using the NBS-19 reference sample).

The brachiopod-bearing facies occurs in West Spitsbergen throughout the entire time interval represented by the Kapp Starostin Formation, but discontinuously in each section. Therefore, we employed the event-stratigraphic time correlation to produce composite curves of changes in the carbon and oxygen isotope ratios (Fig. 3). The productacean samples yield a very clear pattern. The supplementary samples show a little more variation, but they nevertheless largely follow the productacean pattern. As clearly demonstrated by a comparison of Figs 2 and 3, the stratigraphic sequence of carbon isotope measurements taken on samples collected from each particular section is invariably consistent with the overall pattern, so that the most striking features of the pattern are in fact documented within individual sections. This comparison ensures the reliability of our composite curves as an orderly stratigraphic record.

The lower part of the carbon isotope curve from the Kapp Starostin Formation scatters around a $\delta^{13}C$ value of +4‰, which is typical of measurements taken on a variety of Permian rocks from all over the world[5-11,14,29,33]. In the middle part of the section, $\delta^{13}C$ increases rapidly by as much as 3.5‰, a phenomenon recorded also in many other sections[2,5-9,30,34]. The upper part of the curve shows a dramatic decline of $\delta^{13}C$ by more than 10‰, falling to pronouncedly negative values. A distinct drop in the carbon isotope ratio at the P/T transition occurs also in Transcaucasian[2], Chinese[10] and Alpine sections[11], but nowhere has it been observed to be of such extraordinary magnitude. Thus, the carbon isotope curve obtained in West Spitsbergen is generally consistent with all the data reported previously. Given the biostratigraphic data we have, the timespan of the two carbon isotope shifts together is of the order of a few million years. The oxygen isotope curve essentially mimics the carbon curve, although its decline in the uppermost part of the Kapp Starostin Formation is slightly less dramatic, presumably due to a regional cooling[35].

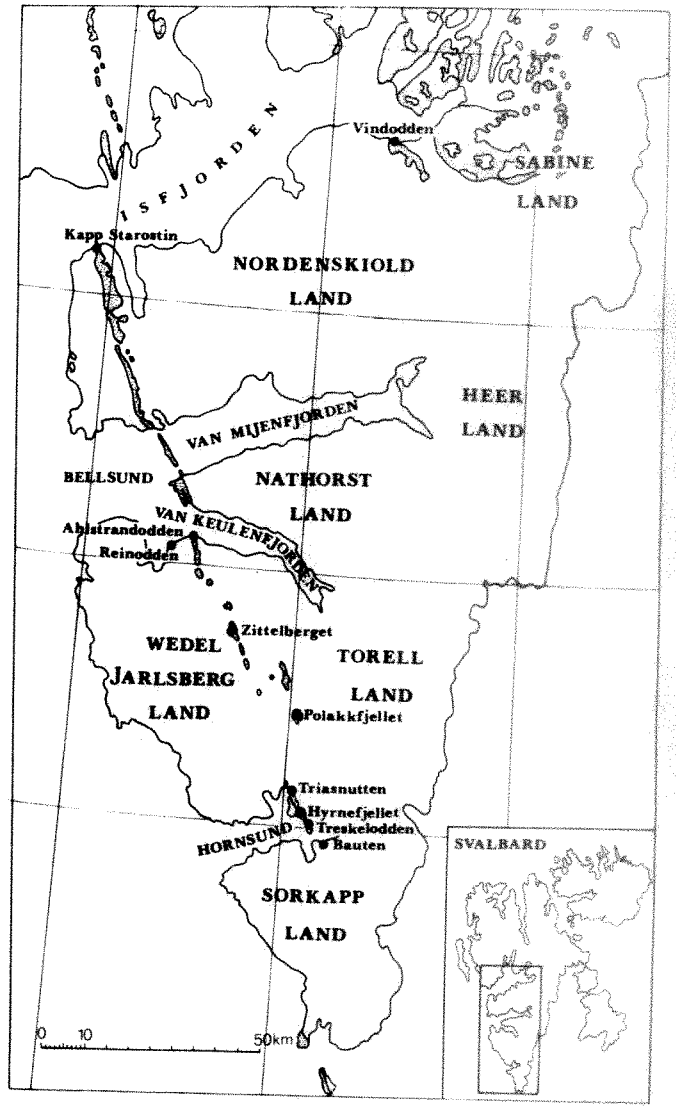


Fig. 1 Location map of the outcrops (shaded areas) of the Kapp Starostin Formation in West Spitsbergen.

To ensure that these carbon and oxygen isotope patterns are not biased by diagenetic alterations, we tested our brachiopod shell material for minor-element proportions. The results of this analysis are presented in Fig. 4 and show that our brachiopod calcite material has not been substantially altered (compare refs 14 and 15); they also show that the samples with extreme $\delta^{13}C$ values, which essentially determine the isotope patterns, do not significantly deviate from the others in this respect. It is worth noting, moreover, that even severe diagenetic changes under the influence of meteoric water have not been reported to effect a change of more than 2–4‰ in $\delta^{18}O$, as compared to the 7‰ shift we observe, and could not possibly lead to the $\delta^{18}O$ values as extreme as −11‰ that we find[36-38]. Having thus ruled out the possibility of diagenetic controls on our empirical pattern, we must interpret the covarying curves of $\delta^{13}C$ and $\delta^{18}O$ as reflecting a global process in the Earth's exosystem.

In this perspective, the magnitude of the carbon isotope shifts is the most extraordinary feature of the pattern. The carbon isotope curve implies that the Late Permian ocean was at first relatively impoverished (spike) and then enriched (drop) in the light isotope of carbon. Relative impoverishment in the light isotope indicates removal of isotopically light organic carbon

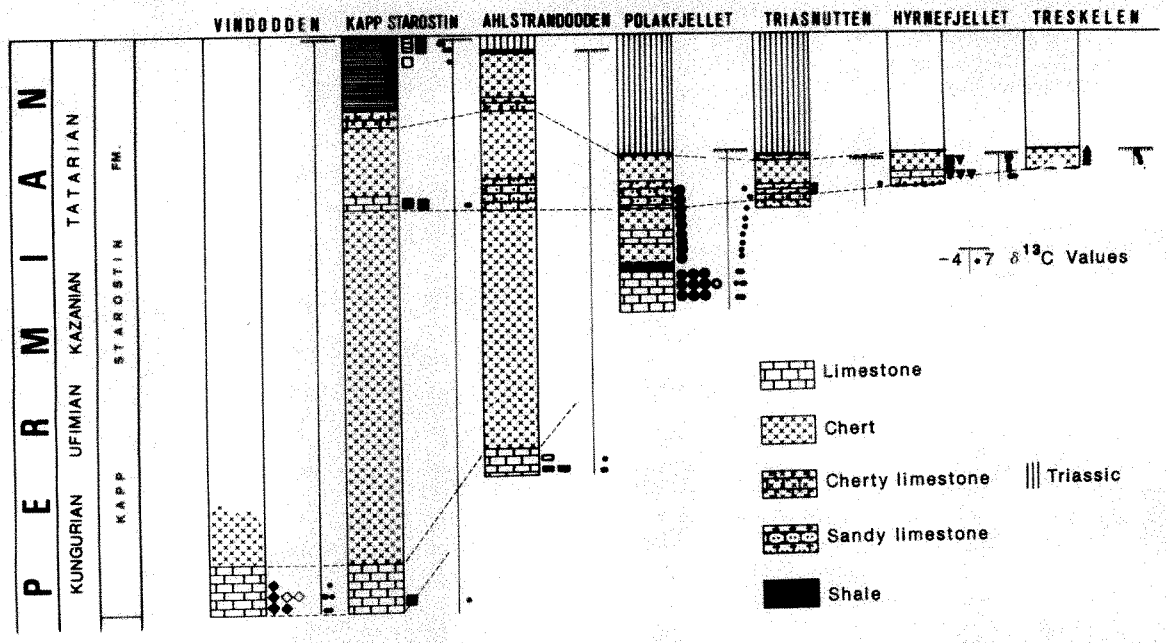


Fig. 2 Time correlation of the investigated sections and sample location; closed symbols denote productacean brachiopod shell material, open symbols indicate other fossil and bulk rock material; $\delta^{13}C$ values for individual samples are shown graphically; bold lines in the bar charts denote the P/T boundary; dashed lines are time correlation lines.

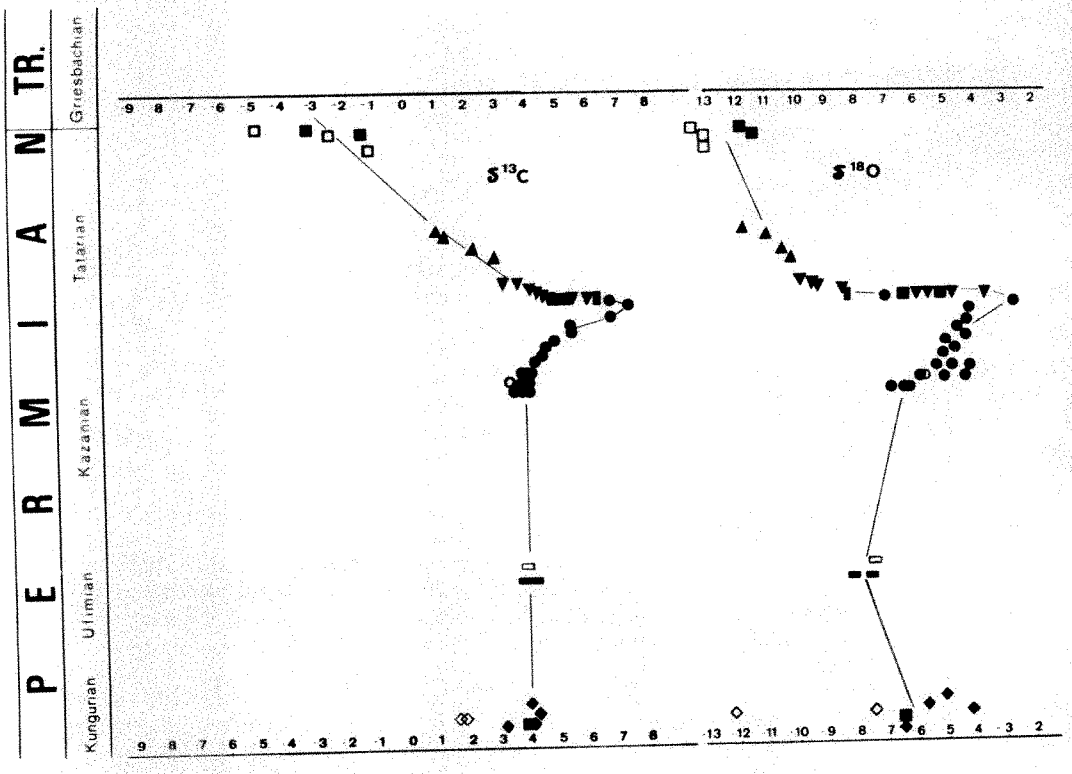


Fig. 3 Carbon and oxygen isotope curves obtained for the Kapp Starostin Formation; sample symbols are as in Fig. 2.

from the ocean-atmosphere system, that is, increased burial rates; relative enrichment, in turn, may be explained either by diminished burial rates of organic carbon, by diminished uptake of the light isotope by the biosphere, or by its increased input to the system, in the form of organic carbon from land ($\delta^{13}C$ of −25‰), marine deposits (−15 to −20‰), or as juvenile, volcanic carbon dioxide ($\delta^{13}C$ of −5‰). Spitzy and Degens[39] have derived a formula to calculate the amount of carbon with a particular isotopic composition that must be supplied to, or removed from, the reservoir with a given isotope ratio in order to cause a specified shift in $\delta^{13}C$:

$$N_B = N_A \cdot \frac{\left[1+\delta_{std}\cdot\left(1+\frac{\delta_B}{1{,}000}\right)\right](\delta_A-\delta_M)}{\left[1+\delta_{std}\cdot\left(1+\frac{\delta_A}{1{,}000}\right)\right](\delta_M-\delta_B)}$$

where N_A is the initial reservoir mass of carbon, N_B is the perturbing mass and δs denote the isotopic compositions in standard notation, with subscripts indicating the initial (A), the

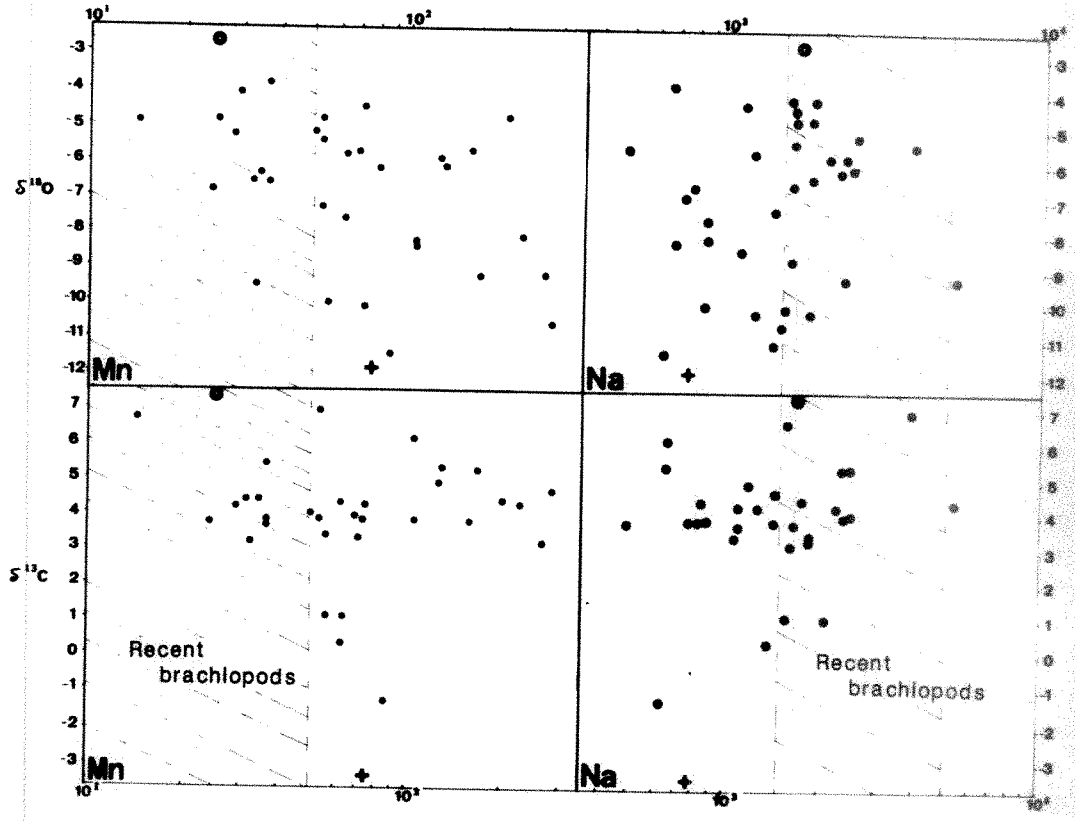


Fig. 4 Manganese and sodium proportions in the productacean shell samples analysed for carbon and oxygen isotopes, as compared with the values typically obtained in Recent brachiopods, show no diagenetic trends in the material (cf. refs 14 and 15); samples with extremely positive (circle) and extremely negative (cross) $\delta^{13}C$ are indicated. Shaded regions include Recent brachiopods.

perturbing (B), the resulting (M) and the reference standard (Std) values. From this equation and assuming that the Late Permian ocean contained as much carbon as the present one (4×10^{13} tonnes), the amount of volcanic carbon necessary to cause the latest Permian drop in $\delta^{13}C$ would demand an increase in juvenile carbon dioxide supply by three orders of magnitude, which is implausible. Hence, organic carbon must have been shifted from one reservoir to another. Assuming that the organic carbon shifted had a $\delta^{13}C$ value of −15‰ and that the Late Permian biosphere contained as much carbon as the present one (5.6×10^{11} tonnes), the positive spike in $\delta^{13}C$ which we observe is equivalent to removal from the ocean-atmosphere system of more than ten times the total amount of carbon in the living biosphere; the subsequent drop in $\delta^{13}C$ is equivalent to input of organic carbon in amounts comparable to the total carbon content in the ocean, or two orders of magnitude more than the total living biomass. Neither a complete cessation of carbon uptake by the biosphere (as a result of its total extinction) nor a dramatic decrease in organic matter burial could account for such a rise in the amounts of isotopically light carbon contained in the ocean.

Hence, the global process reflected by our carbon isotope curve can only be envisaged as increased burial of organic matter (spike) followed by its oxidation (drop). This interpretation is indeed consistent with our oxygen isotope curve, because oxidation of organic matter, reflected by a drop in $\delta^{13}C$, must lead—through kinetic fractionation processes—to a decline in $\delta^{18}O$ as measured in all species of dissolved oxidized carbon[40].

As we deal here with changes in isotope composition of the sea water, the most efficient explanation for our empirical pattern is that both the initial burial and the subsequent oxidation of organic matter occurred in the ocean, rather than in another carbon reservoir. A similar phenomenon took place at the Precambrian/Cambrian transition[41]. This phenomenon can be explained by oxidation of organic matter accumulated previously in the ocean simply because continents were barren at the time and could not yet provide large amounts of organic carbon. Our data thus indicate very high depositional rates of organic matter in the Late Permian ocean, which were replaced at the P/T transition by its extensive oxidation.

This conclusion has tremendous implications for the history of the ocean–atmosphere system and, consequently, life. The increased burial in the ocean must have left much oxygen free. Under the resultant reducing conditions at the sea bottom, phosphorus and nitrogen recycling was enhanced[42]. The subsequent oxidation of organic matter, however, must have drawn much oxygen from the atmosphere, and oceanic phosphorus and nitrogen then sank to the sea floor because of their oxidation[43]. The resulting decline in atmospheric oxygen levels and nutrient deficiency could be the main causes of mass extinction at the P/T transition.

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Peat acidification in Scotland

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Simulation experiments have shown that soils can become more acid when treated with accelerated loads of acid precipitation[1–3]. Field observations, however, give less clearcut answers. Acid deposition is rarely the only variable between sites, and effects are too small for soil *p*H changes to be measurable over much less than a decade. To circumvent these problems, Hallbacken and Tamm[4] in Sweden and Billett *et al.*[5] in Scotland re-sampled soil profiles in forests after 39–57 years and showed that many surface organic and mineral horizons had acidified, but biological acidification and acid deposition may both be implicated in the observed changes[5]. Here we compare peat acidity and base saturation with modelled acid deposition at 123 sites in Scotland. Peats with highest acidity ($pH(CaCl_2) \leq 3.0$) and with lowest base saturation ($\leq 10\%$) are found mainly where deposited acidity was greater than 0.8 kg H^+ ha^{-1} yr^{-1}, providing conclusive evidence that acid deposition plays an important part in the acidification of some soils.

Dystrophic and dystrophic-flushed peats are believed to be particularly susceptible to acid deposition. The surface layers of such peats rely directly upon ion-exchange properties and precipitation for the supply of cations and anions. Acid precipitation increases the leaching of cations from organic soils[3]; exchangeable cations are replaced by H^+ from the incoming precipitation. For soils with only thin organic layers, leached cations may be replaced by biogeochemical weathering in the underlying mineral layers and by element cycling. Ions may be transported upwards from the mineral soil into the organic layer by roots penetrating into the underlying deeper horizons, and also by periodic rises of the water table. In soils such as peat, in which the organic horizons are thick ($\geq$0.5 m), this mechanism works only to a very limited extent[6]. Therefore the surface of dystrophic and dystrophic-flushed peats should reflect deposition chemistry rather than underlying soil mineralogy, and can be a valuable tool in estimating the effects of rain chemistry on peat chemistry.

We have compared the peat chemistry and a model of acid deposition at 123 sites in Scotland. Data on the chemistry of hill peats were extracted from the Scottish National Soil Inventory set up by the Macaulay Land Use Research Institute (Aberdeen). Soil samples for this inventory were collected after 1978. They were air-dried at 30 °C, and 2-mm-sieved samples were stored until analysis, usually within a year. Soils are classified as peat when the organic matter was at least 0.5 m thick. Only dystrophic and dystrophic-flushed peats, collected from sites where the slope form was straight or convex, were included in this study. Most peat samples were collected from the top 15 cm of the peat horizon, some from the top 20 cm and three samples from the top 25 cm. Samples were analysed for *p*H ($CaCl_2$) and percentage of base saturation at the Macaulay Institute, using standard methods. Peat *p*H and base saturation were plotted and their distribution in Scotland was compared with total wet- and dry-deposited acidity calculated for each peat sampling site using the Harwell trajectory model[7]. Deposited acidity at each sampling site was estimated by including contributions from both dry- and wet-deposition routes, and from acidic sulphur (SO_x), nitrogen (NO_x) and chlorine (HCl) pollutants emitted from sources within the United Kingdom and the rest of Europe. Total wet- and dry-deposited acidity was expressed in kg H^+ ha^{-1} yr^{-1}, assuming that 2 g H^+ are equivalent to 32 g S, 1 g H^+ to 14 g N and 1 g H^+ to 35.5 g Cl, and making no adjustment for the deposition of ammonia and its derivatives.

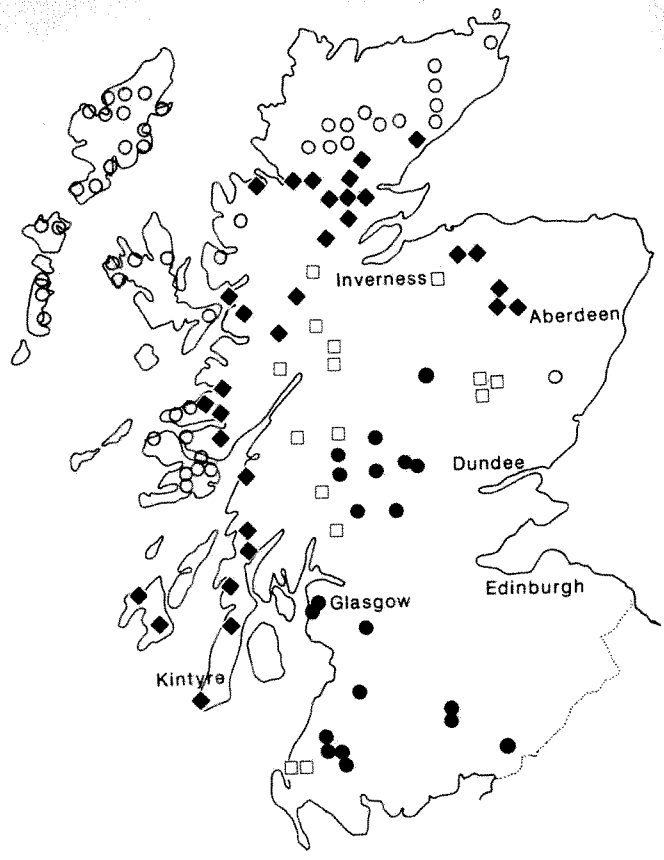


Fig. 1 Modelled wet- and dry-deposited acidity from S, N and Cl species in Scotland (kg H^+ ha^{-1} yr^{-1}). Symbols are: ○, ≤0.6; ◆, >0.6–0.8; □, >0.8–1.0; ●, >1.

The total acid deposition rate in the trajectory model decreased by more than a factor of three across Scotland, from a maximum of >1 kg H^+ ha^{-1} yr^{-1} in south Scotland to a minimum of <0.6 kg H^+ ha^{-1} yr^{-1} in the Outer Hebrides (Fig. 1). Although the prevailing winds throughout Scotland are dominated by winds from the north-westerly through to south-westerly sectors, much of the wet- and dry-deposited acidity in the model is associated with winds and rain from the easterly through to southerly sectors. This reflects the important contribution to deposited acidity from emission sources in England and in the rest of Europe.

Most peats in the areas where acid deposition was >0.8 kg H^+ ha^{-1} yr^{-1} had low *p*H values ($\leq$*p*H 3, Fig. 2) and low base saturation ($\leq$10%, Fig. 3). The correlation coefficients (r^2) for peat *p*H-total acid deposition and for base saturation-total acid deposition were significant, at $p < 0.02$ ($r^2 = 0.051$) for the former and $p < 0.001$ ($r^2 = 0.278$) for the latter.

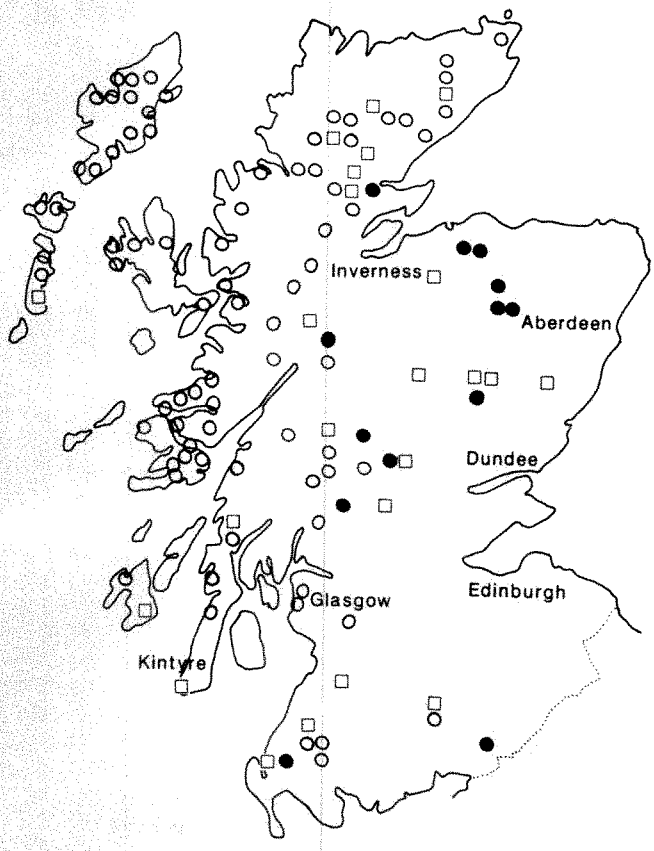


Fig. 2 The pH ($CaCl_2$) of peats in Scotland. ●, ≤2.8 (mean pH of all sites in this group analysed was 2.75); □, >2.8–3.0 (mean pH 2.94); ○, >3.0 (mean pH 3.35).

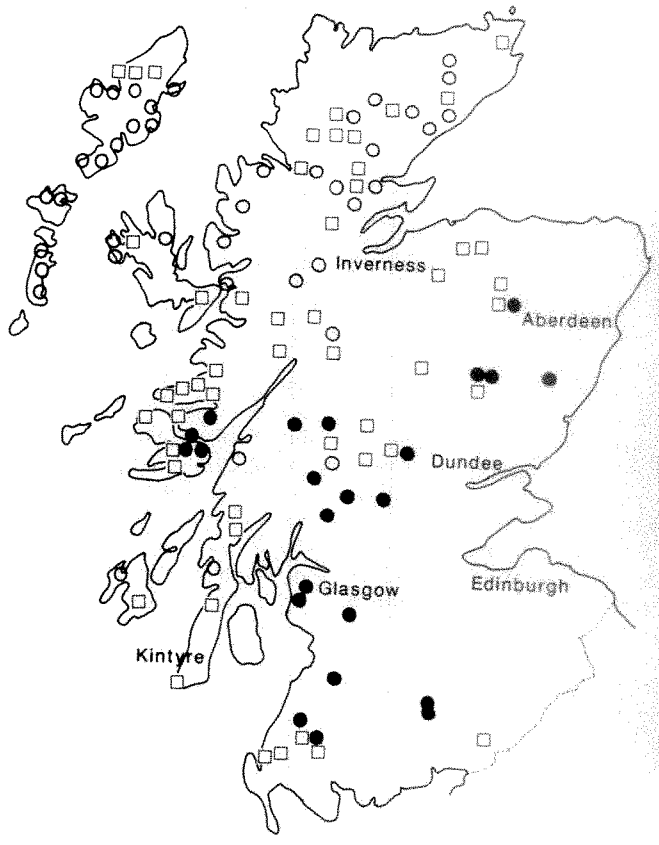


Fig. 3 Percentage base saturation in peats in Scotland. ●, ≤10% (mean % base saturation 6.4); □, >10%–20% (mean % base saturation 15.6); ○, >20% (mean % base saturation 25.1).

Peats were most acidic (pH ≤ 2.8) between Glasgow and Inverness. Acid deposition, however, was greatest in the south, but the number of acid peats sampled here was lower because the peat is less extensive in this area. Land use and management, and position of the peats sampled on a hill site, influence the chemistry of the peat and contribute to the variability in the data observed. Some acid peats (between pH 2.8 and 3.0) were also found on Kintyre and north of Inverness. The spatial distribution of acidifying emissions shows that these pockets of high peat acidity coincided with pockets of SO_2 and NO_x emissions greater than those in surrounding areas[8].

Acid deposition and low base-saturation distributions (Figs 1 and 3) show very good agreement. Low base saturation did not correlate with other variables, such as altitude or rainfall amount. 70% of all peats sampled with a base saturation of <10% came from areas where the modelled wet- and dry-deposited acidity was $>0.8\ kg\ H^+\ ha^{-1}\ yr^{-1}$. On the other hand, 60% of all peats with a base saturation greater than 20% were collected from areas where the deposited acidity was $<0.6\ kg\ H^+\ ha^{-1}\ yr^{-1}$.

The distribution of acid peats and low base saturation of peats in Scotland also correlates well with the recent loss of acid-sensitive lichens. Some lichens, particularly *Lobaria pulmonaria*, are sensitive to low levels of air pollution, primarily SO_2. Distribution maps of *Lobaria pulmonaria* showed that, since 1960, it has disappeared from sites in eastern Scotland, around Edinburgh and Glasgow and also at two sites north of Inverness[9].

As emissions of acid-producing pollutants are declining in many European countries, it is of interest to predict how long it will take for acidified soils to become less acid. A simulation experiment on an accelerated timescale suggests that a decrease in peat acidity should be noticed in a relatively short time period—years rather than decades[3]. Assuming a precipitation pH of 5, extrapolation of published results[3] of simulation experiments showed that a peat now having a pH of 2.8 should decrease in acidity by 0.34 pH units within 10 years, assuming a rainfall of 2,000 mm. After 100 years a decrease in acidity of 0.53 pH units can be expected. The present difference in peat acidity between the south and east of Scotland and the north-west of Scotland is 0.55 pH units (Fig. 2). This suggests that simulated rain experiments yield realistic values for possible pH changes occurring.

The significant correlation between peat acidity or base saturation and total acid deposition gives strong evidence for acid deposition having caused further acidification of peats in Scotland over and above natural levels. As emissions of acid-producing pollutants are declining in many European countries, recovery should become evident in a few decades.

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Female transfer and inbreeding avoidance in social mammals

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In most social mammals, males leave their natal group to breed in other groups whereas females commonly remain in the same group throughout their lives[1]. In a few species however, females usually transfer between groups during adolescence[1]. The functional significance of sex differences in dispersal and their connection, if any, to the avoidance of inbreeding is disputed[2–7]. Here I show that in polygynous mammals where females commonly remain to breed in their natal group, their average age at first conception typically exceeds the average period of residence of adult males in breeding groups. In contrast, where females usually transfer to breed in other groups, the average residence of breeding males or of resident male kin groups typically exceeds the average age of females at first conception. These results support the suggestion that female mammals commonly transfer to avoid inbreeding with their father or other close relatives, although female dispersal may also occur for other reasons[8,9].

Most polygynous, group-living mammals can be classified into three groups on the basis of dispersal. In the majority of species, most males emigrate from their natal group at or shortly after puberty and transfer to other breeding groups, either immediately or at some later stage of their lifespan[1]. Although female emigration is not uncommon in some of these species and female groups may subdivide and occasionally coalesce[3], female transfer into established breeding groups is unusual. Second, in a small number of species females habitually transfer to other breeding groups during adolescence, whereas males commonly remain in their natal group[10–15]. Finally, in a few species that live in uni-male harem groups, both sexes usually disperse from their natal group at adolescence to breed in other groups[16–19].

Male-biased dispersal may predominate in polygynous mammals because adolescent males are commonly ejected from their natal group by resident males[2,3], because the potential benefits of dispersal are usually higher for males than females[20–22] and because the costs of dispersal are generally higher for females because of their lower age at first breeding[7], their more stringent energetic requirements[5,23], and their dependence on coalitions with relatives[24]. Where the majority of males disperse at adolescence, females will seldom need to disperse to avoid inbreeding. So why do females usually disperse in some species? One possible explanation is that females habitually transfer during adolescence in species where the effective breeding lifespans of males within particular groups commonly exceed the age of females at first conception[1,4,21] and where females would risk inbreeding with their father or other close relatives if they remained in their natal group. If so, predominant female dispersal should be associated with male residence in breeding groups that usually exceeds the age of females at first breeding, whereas in species where females remain in their natal group, male residence should usually be shorter than the age at which females breed for the first time.

To test this prediction, I extracted all available estimates of the mean age of females at first conception and of the mean residence of breeding males in particular groups or breeding territories for social, polygynous mammals from the literature. Where several related males defend access to female groups, I used the minimum residence of male kin groups. For one species (grey kangaroos) where a single male is responsible for most matings, I used the average period for which males held alpha rank. The eventual sample included eighteen species where males habitually transfer from their natal group and females usually remain there (Table 1) and nine species where females commonly transfer from their natal group to breed elsewhere (Table 2). The sample of male-dispersing species included ten usually found in uni-male harems (which may include more

Table 1 Social mammals where male transfer predominates

Species	Predominant transferring sex	Mean female age at first conception (months)	Mean male residence (months)	Does female age at first breeding exceed average male residence in a single group?	Refs
Uni-male groups or territories					
Yellowbellied marmot (*Marmota flaviventris*)	M	36	27 R	Yes	29
Blacktailed prairie dog (*Cynomys ludovicianus*)	M	24	<24 R	Yes	27
Red deer (*Cervus elaphus*)	M	40	36 R	Yes	30
Wedge-capped capuchin (*Cebus olivaceus*)	M	72	120 R	No	51
Redtail monkey (*Cercopithecus ascanius*)	M	24	<24 R	Yes	31
Campbell's guenon (*C. campbelli*)	M	36	<34 R	Yes	31
Blue monkey (*C. mitis*)	M	66	<40 R	Yes	31
Gelada baboon (*Theropithecus gelada*)	M	54	43 R	Yes	32
Grey langur (*Presbyties entellus*)	M	36	28 R	Yes	33
Purple leaf monkey (*P. senex*)	M	48	36 R	Yes	34
Multi-male groups					
Eastern grey kangaroo (*Macropus giganteus*)	M	24	12 α	Yes	35
African lion (*Panthera leo*)	M	38	26 R	Yes	24
Ringtail lemur (*Lemur catta*)	M	30	<24 R	Probably	23, 36
Vervet monkey (*Cercopithecus aethiops*)	M	33	32 R	Yes	37
Yellow baboon (*Papio cynocephalus*)	M	72	<51 R	Yes	38
Rhesus macaque (*Macaca mulatta*)	M	66	19 R	Yes	39
Japanese macaque (*M. fuscata*)	M	54	<30 R	Yes	40, 41
Toque macaque (*M. sinica*)	M	60	50 R	Yes	37, 42, 43

For mean male residence: R, residence; α, tenure of α rank. Field data were used to estimate the age of females at first conception wherever possible supplemented by data from ref. 44. Average male residence was the estimated duration of reproductive activity in males within a particular group. In the majority of species, this was equivalent to mean residence per group in non-natal breeding groups but for grey kangaroos I have used the mean tenure of alpha rank and for red deer the average breeding lifespan.

Table 2 Social mammals where female transfer predominates or where both sexes usually transfer

Species	Predominant transferring sex	Mean female age at first breeding (months)	Mean male residence (months)	Does female age at first breeding exceed average male residence in a single group?	Refs
Uni-male groups					
Plains zebra (*Equus burchelli*)	M+F	24	>60 R	No	16, 45
Feral horse (*E. caballus*)	M+F	25	38 R	No	46
Hamadryas baboon (*Papio hamadryas*)	M+F	52	>36 R	No	18*
Mountain gorilla (*Gorilla gorilla beringei*)	M+F	119	180 R	No	19†
Multi-male groups					
Cape hunting dog (*Lycaon pictus*)	M+F	38	Resident male kin group	No	10, 11, 47
Red howler monkey (*Alouatta seniculus*)	M+F	49	71 R	No	8, 48‡
Red colobus monkey (*Colobus badius*)	F	46	Resident male kin group	No	12, 13, 49
Chimpanzee (*Pan troglodytes*)	F	120+	Resident male kin group	No	14, 50
Bonobo (*P. paniscus*)	F	120+	Resident male kin group	Probably not	14, 50

In four species, groups of related males defend access to groups or communities of females. The average residence of male kin groups is unknown in all cases, but take-overs are rare and the average tenure of male groups is evidently long. In one species (hamadryas baboons) females leave their natal units but remain within a larger social unit, consisting of several harems, often belonging to related males.

* Also, H. Kummer, personal communication; † A. H. Harcourt, personal communication; ‡ R. Rudran (unpublished).

than one adult male but where one is responsible for most of all mating) and eight usually found in multi-male, multi-female groups (where more than one mate is involved in breeding). The sample of female dispersing species included four species normally found in uni-male harem groups and four species where a resident kin group of males defends breeding access to a female group. Two further species, black spider monkeys *Ateles paniscus* and woolly monkeys *Lagothrix lagothricha*, may also have breeding systems of this kind but their pattern of dispersal is not yet known[25,26].

In all but one of the eighteen species where females typically remain in their natal group and males usually transfer to breed in other groups, the average age of females at first breeding exceeds the average residence of males in breeding groups (see Table 1). In contrast, in all nine species where females habitually transfer from their natal group, the average residence of breeding males is longer than the age at which females begin to breed (see Table 2). The residence of male kin groups is not known for hunting dogs, red colobus, chimpanzees and bonobos, but take-overs by extra-group males are evidently unusual and it is clear that the average residence of kin groups typically exceeds the age of females at first conception. There is an interesting contrast between these species and lions (see Table 1), where groups of related males also defend breeding access to female prides but take-overs are frequent, male residences are short (average 26 months) and females rarely disperse[24].

The distribution of dispersal thus suggests that where males can effectively defend mating access to feeding territories or to female groups for periods longer than the age of females at first breeding, females may disperse to avoid mating with close relatives. Where male dispersal at adolescence is associated with long male residence (as in gorillas) both sexes are likely to disperse from natal groups. The association between female dispersal and long male tenure does not indicate that females never disperse for other reasons: in at least two social primates, females commonly disperse in circumstances where close inbreeding is unlikely[8,9]. Nor does it indicate that females never risk inbreeding with their fathers or brothers. In some species where females remain in their natal group, the difference between the average duration of male residence and the average age of females at first breeding is small (see Table 1) and a proportion of females will reach breeding age while their father is still active in their group[31]. Under these circumstances, recent evidence suggests that breeding males sometimes disperse or avoid mating with their daughters[5,24,27] or females may disperse[19], attempt to mate with extra-group males[24] or delay conception[28].

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Pointillistic mixing of interference colours in cryptic tiger beetles

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In insects and other animals, interference colours usually serve as conspicuous visual signals[1-4]. The colours of tiger beetles also originate by interference of multiple reflections[5,6], yet many species exhibit dorsal colorations that appear pigmentary and inconspicuous (cryptic) against soil backgrounds. These dull colours, however, are actually additive mixtures of different interference colours that blend when viewed with the naked eye. This same principle of partitive mixing is used in colour television, printing and the pointillist painting style of neo-impressionist artists[7]. We report here on the first demonstration of interference colours mixing partitively to produce animal camouflage.

Ultrastructural studies show that tiger beetle colours originate from a series of thin cuticular layers near the outer surface of the integument[5]. These layers function optically as a multilayered interference filter. The layers lie directly above a thicker melanized region and conform to a surface microsculpture consisting of alveoli (hexagonal pits) that measure 13 μm across (Fig. 1). The reflectance spectrum depends on the optical thicknesses of the layers and the angles of illumination and observation[8].

We examined the elytra (wing covers) from two colour morphs of the tiger beetle *Cicindela oregona* to determine how interference reflections produced their cryptic colorations. As in many cicindelids[9,10], the elytral coloration of *C. oregona* varies geographically and matches the local soil colour[11]. The morphs exhibited brown or black elytra that appeared pigmentary to the unaided eye. When viewed through an incident-light microscope, however, the alveolar basins and ridges produced a mosaic of coloured reflections that changed with viewing angle. Moreover, discrete patches of alveoli (40-80 μm across) reflected interference colours different from those of the surrounding alveoli.

To compare colours at the macro- and microscopic levels, we measured reflectance spectra for small fields of uniform colour and larger fields that contained the different colour patches. Reflectance spectra from each colour morph were created by mounting specimens of *C. oregona* on the universal stage of an incident-light microspectrophotometer, focusing on an area representative of the elytral colour, and measuring the reflectance with monochromatic flashes at 10 nm intervals[12].

The brown morph exhibited circular patches of blue-green

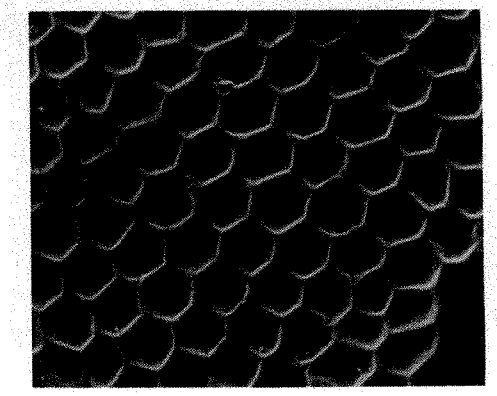


Fig. 1 Scanning electron micrograph of the elytral surface of *Cicindela oregona*. The microsculpture consists of small hexagonal alveoli that measure 13 μm across (magnification ×600).

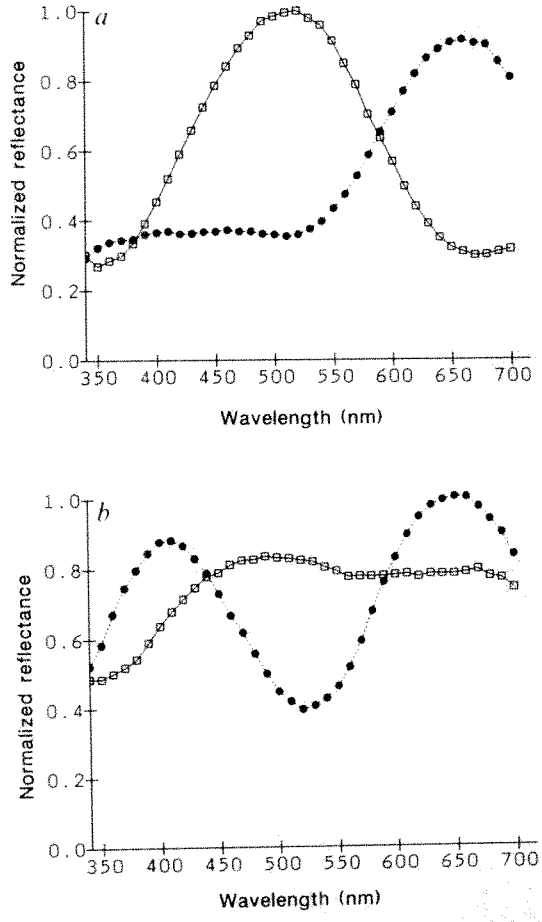


Fig. 2 Normalized reflectance spectra for adjacent microscopic colour patches on the elytra of *Cicindela oregona* measured under high power through a ×40/0.75 objective. Reflectances were measured for patches 30-40 μm in diameter containing 5-8 alveoli of the same colour. The brown morph (*a*) contained blue-green patches (—⊟—) embedded in a red field (···●···). The black morph (*b*) exhibited irregular magenta patches (···●···) surrounded by dull green areas (—⊟—). The microspectrophotometer was based on a Leitz Ortholux microscope with MPV Pol-Opak illuminator, ×8/0.18P or Nikon CF Fluor ×20/0.75 objectives, and Zeiss UV-projective coupled to a Hammamatsu R928 photomultiplier. The aperture stop of the Epi-illuminator was reduced to near minimum, and the incident angle was made as oblique as possible. The instrumental baseline was calibrated with a National Bureau of Standards SRM-2003D spectral reflectance standard.

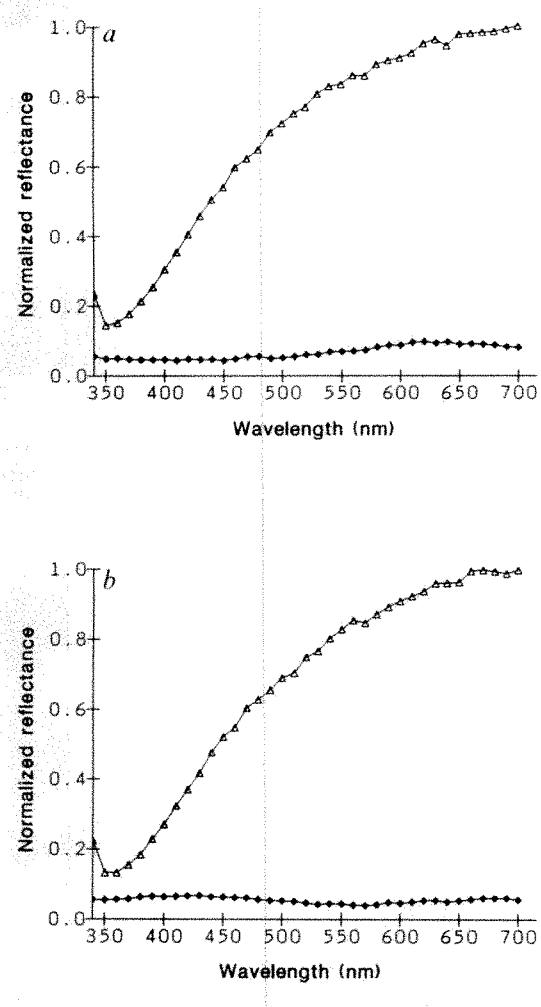


Fig. 3 Normalized reflectance spectra of coloured and white elytral areas from the same two morphs of *C. oregona* but measured under low power through a ×8/0.18 objective. Reflectances were measured, for fields (300 μm in diameter) containing ~530 alveoli, in both the coloured and white areas of the brown (*a*) and black (*b*) elytra. Fields in the coloured area contained 7–8 microscopic colour patches described in Fig. 2. Reflectances of the coloured areas (—◆—) are plotted with the same normalization constant as the white areas (—△—) to illustrate the desaturation and low brightness of the mixed colours.

alveoli surrounded by a red field (Fig. 2*a*). In the black morph, irregular patches of magenta interference colours were surrounded by dull green alveoli that reflected both green and magenta colours (Fig. 2*b*). The alveoli of pure magenta patches produced two prominent reflectance peaks at 660 nm and 400 nm.

Spectra produced by partitive mixing of the microscopic colour patches were measured by decreasing the magnification to include eight colour patches and their surrounding field. The resulting spectra were low in intensity and saturation when compared to an adjacent white elytral spot. The brown morph showed a broad, and slightly elevated reflectance between 500 and 750 nm due to the summation of green and red reflections (Fig. 3*a*). In the black elytra, the summation of green and magenta areas produced a relatively flat, unsaturated reflectance spectrum (Fig. 3*b*).

In tiger beetles such as *C. oregona*, reflecting layers may vary in periodicity, resulting in small clusters of alveoli that reflect different wavelengths[5]. These colour patches are too small to be resolved by the unaided eye and spatially fuse to produce an additive colour mixture[13]. Partitive mixing of different spectral combinations produces different cryptic colour morphs of *C. oregona*.

In addition, the surface microsculpture affects the intensity and wavelength content of these mixed colours. The pitted elytral surface scatters the reflected light over a wide range of angles, thereby reducing the amount of light measured by the modest collecting angle of the microscope. Similarly, much of the scattered light escapes the even smaller collecting angle of the observer's eye, causing the colours to appear dark. The reflectance spectrum is affected also by the angle of the reflecting surface relative to incident light[8]. Shorter wavelengths reflected from the angled walls of the alveoli additively mix with those reflected from the ridges and basins, reducing the purity of the colour.

Many cryptic colorations of animals are produced through partitive mixing, but most involve pigments[14,15]. However, in tiger beetles and other Coleoptera[16,17], partitive mixing of different interference colours and scattering by the surface microsculpture produce broad-band reflectance spectra that match background colours and serve as camouflage.

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Substitution at residue 227 of H–2 class I molecules abrogates recognition by CD8-dependent, but not CD8-independent, cytotoxic T lymphocytes

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The CD8 (Lyt 2) molecule is a phenotypic marker for T lymphocytes that recognize and react with major histocompatibility complex (MHC) class I molecules. Antibody blocking experiments[1,2] and gene transfection studies[3,4] indicate that CD8 binds to a determinant on MHC class I molecules on the target cells, facilitating interaction between effector T lymphocytes and the target cell. The CD8 molecule may also be involved in transmembrane signalling during T-cell activation[5,6]. The existence of CD8⁻ cytotoxic T lymphocytes (CTL)[7] and class I-reactive CTL that are not inhibited by antibody to CD8 suggests that at least some CTL do not require the CD8 molecule to interact with and lyse target cells. We have recently demonstrated that cells transfected with

an *H-2D*^{d} gene that carries a mutation at residue 227 are not killed by primary CTL[8]. Here we show that although this mutation abrogates recognition by primary CTL, it does not affect recognition by CD8-independent CTL, suggesting that residue 227 of class I molecules might contribute to a determinant that is the ligand of the CD8 molecule.

We first described a somatic cell mutant isolated by immunoselection that expressed a variant H-2D^d molecule which was not killed by alloreactive anti-H-2D^d CTL[9]. This cell line had been isolated because of its failure to express a serological determinant expressed in the α3 domain. Recently[8] we identified the mutation in the α3 domain of the H-2D^d molecule as a Glu → Lys substitution at residue 227. This change was reproduced in a cloned *H-2D*^{d} gene by oligonucleotide-directed mutagenesis. Cells transfected with this mutant gene expressed all the serological epitopes on the H-2D^d α1/α2 domains but were not killed by anti-H-2D^d CTL populations generated *in vitro* in primary responses. This was not apparently a result of the destruction of the conformation of the H-2D^d molecule, because the mutant stimulated an anti-H-2D^d reactive IL-2-secreting T-cell hybridoma as efficiently as the wild-type H-2D^d molecule.

We first determined whether this mutation in other class I genes could abrogate CTL recognition by the creation of hybrid genes through exon shuffling[10,11]. Hybrid genes comprised the 5′ region and exons 1-3 (α1/α2 domains) from the *H-2K*^{b} gene ligated to exons 4-8 (α3 domain, transmembrane and cytoplasmic regions) of the *H-2D*^{d} gene (Fig. 1). One gene, referred to as *H-2K*^{b}/*D*^{d} Glu, had glutamic acid at position 227 (as does wild-type *H-2D*^{d}), whereas the other gene, *H-2K*^{b}/*D*^{d} Lys contained a mutation that leads to substitution with lysine at position 227. Both of these genes were transfected into the cell line R8.161, an H-2^{b-} variant isolated from the R8 (H-2^b/H-2^d) cell line. Cells expressing these genes were isolated and serological expression of H-2K^b α1/α2 epitopes, defined by a panel of antibodies to H-2K^b, was confirmed by flow-microfluorimetry analysis (Fig. 2). The reactivity of antibodies to the α1/α2 domains of H-2K^b with the transfectants expressing either the *H-2K*^{b}/*D*^{d} Glu or *H-2K*^{b}/*D*^{d} Lys genes was identical, indicating that this substitution in the α3 domain did not significantly affect the conformation of the α1/α2 region of H-2K^b. These cells were also used as targets for anti-H-2K^b

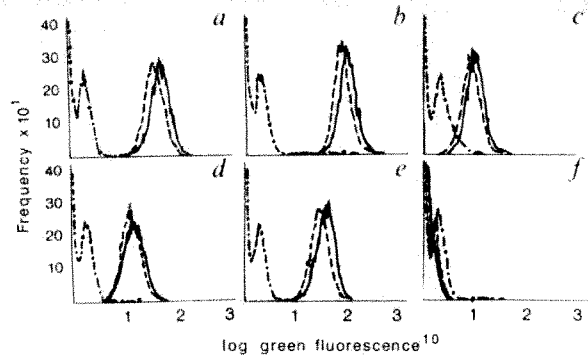


Fig. 2 Flow-microfluorometry analysis of *H-2K*^{b} expression on R8.161 and transfected cell lines.
Methods. Cells were analysed for serological expression of *H-2K*^{b} epitopes by incubating 10^6 cells with 100 µl H-2K^b antibodies at an optimal dilution on ice for 30 min. The cells were washed and then incubated for a further 30 min with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin. After staining, the cells were washed and the fluorescence of 10,000 viable cells measured on an EPICS 752B. The fluorescence profiles for R8.161 H-2K^b Glu (----), R8.161 H-2K^b Lys (——) and R8.161 (-·--·) are shown. The determinants recognized by the EH144 (*a*) and B8-24-3 (*b*) antibodies have been mapped to the α1 domain, antibodies 5F1 (*c*) and 28-13-3 (*d*) to the α2 domain; residues from both α1 and α2 contribute to the determinant recognized by the K9-178 (*e*) antibody[11,16]. Antibody 128-3-3 (*f*) is specific for the H-2K^{bm10} mutation and is used here as a non-reactive control.

CTL generated in a primary *in vitro* response (B10.D2) (ddd) anti-B10 (bbb). These CTL lysed cells expressing the *H-2K*^{b}/*D*^{d} Glu gene product (Fig. 3*a*), but did not lyse transfectants expressing the product of the *H-2K*^{b}/*D*^{d} Lys gene (Fig. 3*d*). This reactivity was similar to that of anti-H-2D^d CTL with cells expressing the mutant or wild-type *H-2D*^{d} gene[8]. Adding antibody to CD8 inhibited the reactivity of the CTL for cells expressing the *H-2K*^{b}/*D*^{d} Glu gene product (Fig. 3*a*). To find out the role of CD8 in the lysis of these cells, we generated CTL that were less readily inhibited by anti-CD8 antibody by priming B10.D2 mice with EL4 cells, followed one week later by restimu-

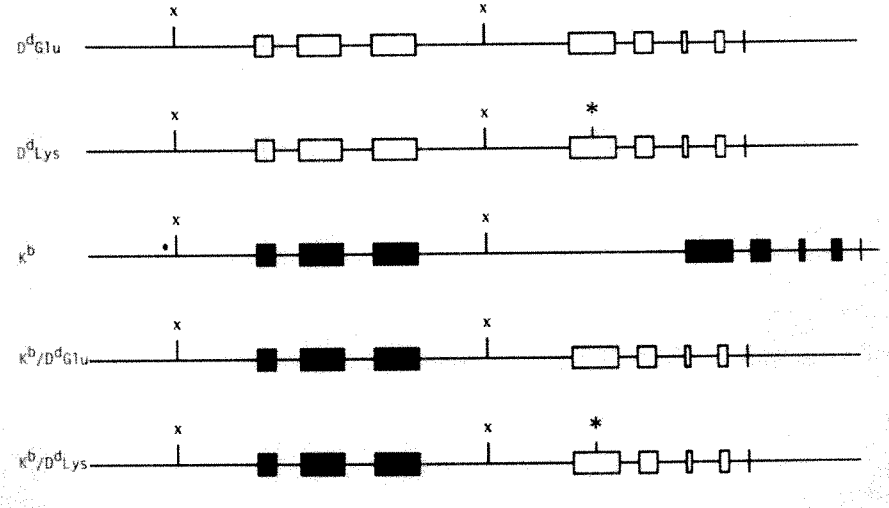


Fig. 1 Construction of hybrid genes used in this study. The *H-2D*^{d} gene used here actually contains a 700-base pair deletion from the *Kpn*I to *Hpa*I site in the third intervening sequence of the *H-2D*^{d} gene isolated by Margulies *et al.*[14]. This deletion, which was produced during the religation of the 2.1-kilobase (kb) *Hpa*I fragment containing exons 4-8 which had been subcloned into M13mp19 for site-directed mutagenesis, did not affect expression of the gene[8]. The mutation at residue 227 was produced by oligonucleotide-directed mutagenesis as previously described[8]. The *H-2K*^{b}/*D*^{d} Glu gene was produced by replacement of the 1.9-kb *Xba*I fragment encoding exons 1-3 of the *H-2D*^{d} gene with a similar fragment from the *H-2K*^{b} gene. Digestion with *Pvu*II ensured that exons 1-3 were inserted in the correct orientation. The hybrid genes were cloned into the pSV2Neo plasmid. Plasmid DNA containing these genes was introduced into R8.161 by electroporation[15]. After 48 hours incubation at 37 °C, G418 antibiotic (Gibco) was added to a concentration of 1.0 mg ml^{-1}. *X* denotes the location of the *Xba*I sites that flank exons 1-3 of both *H-2D*^{d} and *H-2K*^{b} and * denotes the location of the point mutation encoding lysine at residue 227 in the α3 domain.

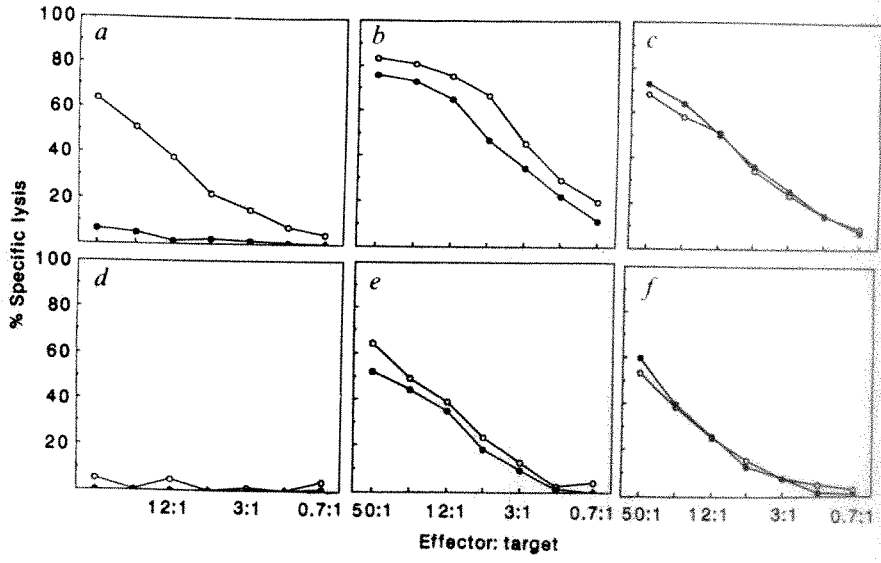


Fig. 3 Reactivity of primary and secondary anti-H-2K^b CTL for the R8.161 cells transfected with the *H-2K^{b}D^d* hybrid genes. The target cells were R8.161 H-2K^b/D^d Glu (*a*–*c*) and R8.161 H-2K^b/D^d Lys (*d*–*f*). The CTL populations were primary anti-H-2K^b (*a* and *d*), secondary anti-H-2K^b generated in the presence of anti-CD8 antibody (*b* and *e*) and primary anti-H-2^d (K^d, D^d, L^d) (*c* and *f*). The assay for CTL reactivity was performed either in the absence (○—○) or the presence (●—●) of an antibody to CD8.2 83-12-5. The inclusion of the anti H-2K^d/D^d CTL population (AKR anti B10.D2; panels *c* and *f*) demonstrated that both types of transfected cells were readily killed by CTL reactive with H-2^d molecules present on transfected and on non-transfected cells. The failure of the anti-CD8.2 antibody to inhibit these CTL, isolated from AKR mice which are CD8.1, established that the effect of this antibody on CTL populations isolated from B10.D2 was specific for the CD8 molecule.

Methods. The primary CTL were generated by a 5-day culture of B10.D2 spleen cells with irradiated (2,500 rad) B10 spleen cells, as previously described[17,18]. 4×10^6 splenic responder cells were mixed with 4×10^6 stimulator cells in a final volume of 2 ml culture medium which contained selected fetal bovine serum (FBS) and 10% lectin-free concanavalin A-induced supernatant, and incubated at 37 °C for 5–6 days. The secondary CTL were generated by priming B10.D2 mice with irradiated (5,000 rad) EL4 tumour cells injected intraperitoneally one week before stimulation of the spleen cells *in vitro* in the presence of CD8.2 (83-12-5) antibody. The reactivity of CTL was assessed by ^{51}Cr release, using 5×10^3 labelled target cells in a final volume of 200 μl. Effector and target cells were incubated together in 96-well U-bottom plates for 4–6 h at 37 °C. Culture supernatants were collected and specific lysis determined as specific release (%): 100 × (experimental release) − (spontaneous release)/(maximum release) − (spontaneous release). The experimental release represents the release of ^{51}Cr from target cells incubated with effector cells. The spontaneous lysis is the release in the absence of effector cells and the maximum release is that released by the target cells in the presence of 0.05 M HCl.

lation of the spleen cells *in vitro* for 5 days in the presence of antibody to CD8. These secondary CTL killed not only the *H-2K^b/D^d* Glu transfectant (Fig. 3*b*), but also the *H-2K^b/D^d* Lys transfectant (Fig. 3*e*). The level of lysis of the *H-2K^b/D^d* Lys transfectant was slightly less than that of the *H-2K^b/D^d* Glu transfectant, but a comparable difference was also found in the lysis of the transfectants by anti H-2^d CTL (Fig. 3*c* and *f*). Although the response of the secondary anti-H-2K^b CTL population was less susceptible than the primary anti-H-2K^b CTL to the addition of antibody to CD8, lysis of the *H-2K^b/D^d* Lys transfectant by the secondary CTL population was partially inhibited by the CD8 antibody (Fig. 3*e*). This effect could be due to the anti-CD8 antibody interfering either with the reaction of CD8 and other class I molecules on the surface of the transfected cells or with events such as receptor redistribution (capping) or signal transduction by the CD8 molecule.

Our interpretation of these findings is that substitution of lysine for glutamic acid at residue 227 destroys the determinant on the H-2 class I molecule that is recognized by the CD8 molecule on CTL. Although we think it is more likely that residue 227 contributes directly to the determinant recognized by CD8, we cannot rule out the possibility that substitution at residue 227 might indirectly alter the determinant bound by CD8. Another possibility is that the mutation at residue 227 could affect the conformation of the $\alpha 1/\alpha 2$ determinants, so that only high-affinity CD8-independent CTL can recognize the molecule. If there were such a major conformational change, however, then at least some of the anti H-2K^b $\alpha 1/\alpha 2$ monoclonal antibodies would probably have reacted differently with the *H-2K^b/D^d* Lys transfectant. As shown here, the antibodies reacted identically with both *H-2K^b/D^d* transfectants.

The three-dimensional structure of an HLA class I molecule has recently been determined[12,13] and indicates that residues from the $\alpha 1$ and $\alpha 2$ domains could form a binding site for antigenic peptides between two α-helices. Residue 227 in the $\alpha 3$ domain is located on the surface of the molecule, is accessible to solvent and is at least 50 Å away from the $\alpha 1/\alpha 2$ peptide-binding cleft (M. A. Saper and D. Wiley, personal communication), suggesting that substitution at position 227 is unlikely to affect CTL determinants in the $\alpha 1/\alpha 2$ domains dramatically. Another implication of our results is that, should CD8 bind to a conserved determinant which includes or is influenced by residue 227, then for CD8-dependent CTL, this interaction must be with the same class I molecule as is interacting with the T-cell receptor α/β complex. This is because the reactivity of primary CD8-dependent anti-H-2K^b CTL was abrogated by the mutation, even though the transfected cells expressed other class I molecules such as H-2K^d, H-2D^d and H-2L^d.

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Duchenne muscular dystrophy gene product is not identical in muscle and brain

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder resulting in progressive degeneration of the muscle. It affects about 1 in 3,500 male children. Becker's muscular dystrophy is a less severe disease allelic to DMD. Some 30% of DMD patients suffer from various degrees of mental retardation (see ref. 1 for review). The giant DMD gene spans about 2,000 kilobases and codes for a 14-kilobase messenger RNA and a protein of molecular weight 427,000 (ref. 2). DMD mRNA is most abundant in skeletal and cardiac muscle and less so in smooth muscle[3–5]. We reported that the expression of the gene is developmentally regulated during the differentiation of primary muscle cultures and in myogenic cell lines in a way similar to the expression of muscle-specific genes such as myosin light chain 2 and skeletal muscle actin[5]. Similar results have been obtained with human primary myogenic cells[6]. Significant levels of DMD mRNA are found in brain tissue[5,7]. Here we show that the transcript of the DMD gene and the amino terminal of the encoded protein differ in brain and muscle. The 5′ ends of these mRNA species are derived from different exons. The results suggest that the two mRNA types are transcribed from different promoters.

To investigate whether the DMD gene product is identical in muscle and brain, we prepared RNA probes complementary to various parts of the DMD mRNA. These RNA probes are described schematically in Fig. 1*a*. Total RNA preparations from rat skeletal muscle, cardiac muscle, aorta, brain and other tissues were assayed with these probes, using the RNase protection method[8]. Figure 2 shows how probes *B* and *C*, which are complementary to nucleotides 2,502–2,588 and 3,680–3,758 respectively of the reported mouse muscle DMD complementary DNA sequence[4,9], are protected by muscle and brain RNA, whereas probe *A*, which is complementary to the first 22 codons of the muscle mRNA, is protected by muscle mRNA and not by brain mRNA.

These results indicate that the 5′ region of the muscle DMD mRNA contains sequences that are not present in the brain mRNA, so we constructed additional probes complementary to sequences in this region of the muscle DMD mRNA (Fig. 1*b*) and assayed them with muscle and brain RNA preparations. Probe *D′* was derived from a mouse genomic clone and is complementary to a sequence extending from the *Mbo*II site in the 5′ noncoding region of the muscle type mRNA (nucleotide 80) into the 5′ flanking region. A major 112-nucleotide-long fragment of this probe is protected by muscle RNA but not by brain RNA. These results were obtained with both rat and mouse mRNA. As the protected fragment is longer than expected (80 nucleotides), we suggest that the 5′ end of the mRNA is located 32 nucleotides upstream from the position deduced from the published sequences of the cDNA clones[4,9]. This conclusion is supported by primer extension experiments, and is in accordance with the position of a putative ATA box located 30 nucleotides upstream from the proposed major cap site (data not shown). Probes *E* and *F* cover codons 24–47 and 58–81 respectively and are protected by both muscle and brain RNA (Fig. 3; the results obtained with probe *F* are not shown).

On the basis of these results and of the proposed structure of the 5′ region of the DMD gene (Fig. 1*b*), we concluded that the sequence of the exon that constitutes the 5′ end of the muscle type DMD mRNA (deduced from the sequences of the muscle cDNA and genomic clones) is not represented in brain DMD mRNA. This exon contains the 5′ untranslated region and the first 11 codons. The protection of probes *E* and *F* by both brain and muscle mRNA indicates that the brain DMD mRNA contains codons 24–47 and codons 58–81 of the muscle type mRNA.

To determine the exact point of divergence between the muscle and brain DMD mRNAs, we constructed a rat-brain cDNA library using an oligonucleotide primer complementary to nucleotides 554–582 of the muscle DMD mRNA. The library was screened with the probes described in the legend to Fig. 4 and a positive clone was purified, amplified and sequenced. Comparison of the rat brain and mouse muscle DMD cDNAs (Fig. 4) reveals an almost complete identity of the sequences starting at the 5′ end of the muscle second exon (small sequence differences, which are all but one silent, are probably a result of the variability between rat and mouse). But sequences 5′ to that point are completely different. Thus, the divergence point between the muscle and brain DMD mRNA types is at the junction between the first and second exons. This divergence also results in a difference between the amino terminal of the brain and muscle dystrophin proteins. The first eleven amino

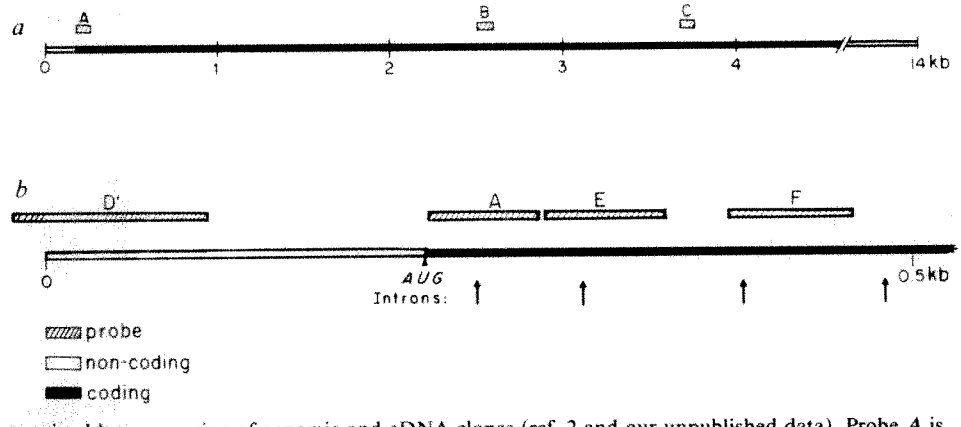
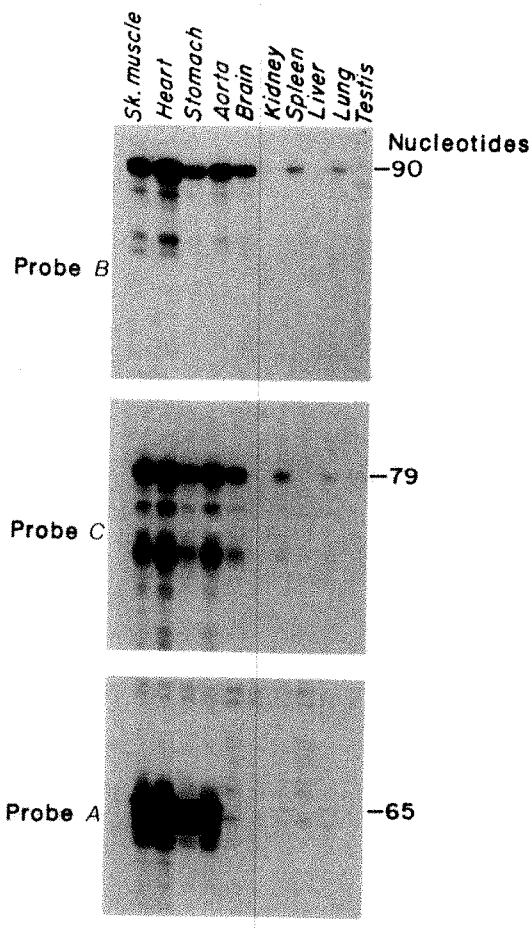


Fig. 1 Schematic representation of the probes used in the RNase protection assay. *a*, The regions of DMD mRNA complementary to probes *A*, *B* and *C*. *b*, The 5′ end of the muscle DMD mRNA and the probes used to analyse this region. The horizontal bar represents the DMD mRNA (blank, noncoding region; solid, coding region). The AUG indicates the putative translation initiation codon. The nucleotide numbering is according to the mouse cDNA sequences reported by Hoffman *et al.*[4] and Koenig *et al.*[9]. Hatched boxes represent regions of mRNA complementary to the probes. Arrows (in *b*) indicate the position of introns as determined by sequencing of genomic and cDNA clones (ref. 2 and our unpublished data). Probe *A* is complementary to nucleotides 221–285 (the putative first 22 codons); probes *B* and *C* are complementary to nucleotides 2,502–2,588 and 3,680–3,758 respectively. Probe *D′* extends from the *Mbo*II site at nucleotide 80 into the 5′ flanking region. Probes *E* and *F* are complementary to nucleotides 289–360 and 395–466 respectively. Probes *A* (pMD12), *C* (pMD51), *E* (pMD61) and *F* (pMD62) were constructed by introducing synthetic oligonucleotides into the vector gemini 3 (Promega). Probe *B* (pMD1), as described previously[5], contains a rat genomic DNA fragment cloned in gemini 3. Probe *D′* (pMD13) contains a mouse genomic DNA fragment cloned in gemini 3. The RNA probes were synthesized using the T7 (probes *A*, *C* and *D′*) or SP6 (probes *B*, *E* and *F*) RNA polymerases.



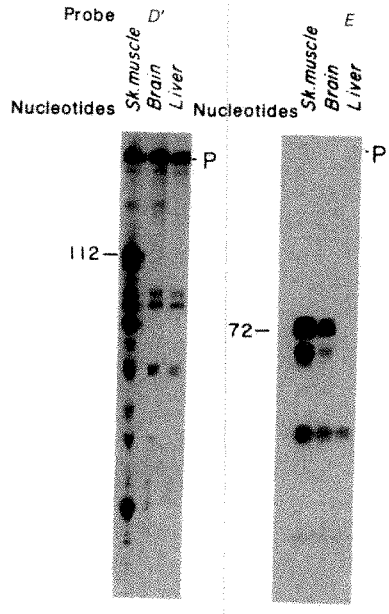


Fig. 3 Differential expression of sequences from the 5′ end of the DMD mRNA in muscle and brain.
Methods. Total RNA (10 μg) from the rat tissues indicated were assayed with probes *D′* and *E* as described in Fig. 2, except that the RNase digestion after the hybridization with probe *D′* was for 1 h at 25 °C. P indicates the position of the undigested probes on the gel, and the numbers indicate the sizes of the main protected fragments. The smaller specific bands are probably caused by unstable regions in the RNA–RNA hybrids.

◀ **Fig. 2** DMD gene expression in various tissues.
Methods. Total RNA was prepared from the tissues indicated using the lithium chloride/urea extraction procedure[11]. The integrity and quantity of all RNA samples were verified by electrophoresis on agarose/formaldehyde gels (not shown). All the RNA samples were extracted from rat tissue, except for testis RNA which was extracted from a mouse. As described previously[5], there is a mismatch between the rat and the mouse sequences at the end of the sequence protecting probe *B*, giving a smaller protected band when mouse RNA is used, as can be seen for testis RNA in the middle panel. The RNA protection assay was as previously described[5], except that hybridization was at 48 °C and 10 μg total RNA were used in each assay. Gels were fluorographed for four days after electrophoresis. *A*, *B* and *C* specify the probe used in each assay. As probes *A* and *C* contain vector sequences and probe *B* includes introns, the protected fragment is shorter than the labelled probe in all cases. The region of the gels containing the protected fragments is shown.

```
Brain:  GACTTCTGGTTCCCAGCAGCCGGCAGTAATAGAATGCTTTCAGGAAGATG  50
Muscle: TGTCAAGGCTGCTGAAGTTTATTGGCTTCTCATCGTACCTAAGCCTCCTG 150

        ACAGAATCAGGAGAAAGATGCTGTTTTGCGCTATCTTGATTTGTTACAGC  100
        GAGCAATAAAACTGGGAGAAACTTTTACCAAGATTTTTATCCCTGCCTTG  200

        AGCCAACTTATTGGCATGATGGAGTGACAGGAAAAACAGCTGGCATGGAA  150
        ATATATACTTTTTTTTCCAAATGCTTTGGTGGGAAGAAGTAGAGGACTGT  250
                              exon 2
        GATGAAAGAGAAGATGTTCAAAAGAAAACATTCACAAAATGGATAAATGC  200
        TATGAAAGAGAAGATGTTCAAAAGAAAACATTCACAAAATGGATAAATGC  300

        ACAATTTTCTAAGTTTGGAAAGCAGCACATAGACAACCTCTTCAGTGACC  250
        ACAATTTTCTAAGTTTGGAAAGCAACACATAGACAACCTCTTCAGTGACC  350
                              exon 3
        TGCAGGATGGGAAACGCCTCCTGGACCTCCTGGAAGGCCTGACAGGCAA  300
        TGCAGGATGGAAAACGCCTCCTAGACCTCTTGGAAGGCCTTACAGGGCAA  400
                              exon 4
        AAACTGCCAAAAGAAAAAGGATCTACAAGAGTTCATGCCCTGAATAATGT  350
        AAACTGCCAAAAGAAAAGGGATCTACAAGAGTTCATGCCCTGAACAATGT  450

        CAACAAGGCACTGCAGGTCTTACAGAAAAATAATGTTGATTTAGTAAATA  400
        CAACAAGGCACTGCGGGTCTTACAGAAAAATAATGTTGATTTAGTGAATA  500
                              exon 5
        TAGGAAGCACTGACATAGTGGATGGAAATCATAAACTCAC            440
        TAGGAAGCACTGACATAGTGGATGGAAATCATAAACTCAC            540
```

Fig. 4 Comparison of the 5′ end region of the brain and muscle type DMD cDNAs. The rat brain DMD cDNA sequence was aligned with the mouse muscle DMD cDNA sequence. The triangles indicate the exon borders (ref. 2 and our unpublished data). The initiator ATGs of the two cDNAs are underlined. The brain type DMD mRNA contains five additional ATG triplets located upstream from the initiator ATG but they do not fit with the open reading frame of the coding sequences. These ATG triplets may be involved in the regulation of translation of the mRNA[16].
Methods. The brain cDNA library was constructed using polyadenylated RNA from rat brain and an oligonucleotide primer extending from nucleotide 554 to nucleotide 582 of the mouse muscle DMD cDNA[9]. The first and the second strands of the cDNA were synthesized using AMV reverse transcriptase and *E. coli* DNA polymerase respectively. After treatment with T4 DNA polymerase to produce blunt ends, ligation with *Eco*RI site-containing linkers, restriction with *Eco*RI and removal of the primer, the cDNA was cloned into the *Eco*RI site of λGT11. Approximately 50,000 phages were screened using two oligonucleotides homologous to the coding region of the mouse muscle DMD cDNA (nucleotides 318–360 and nucleotides 247–300). A phage that hybridized to both probes was purified and amplified. The 500-bp DNA insert was cloned into the *Eco*RI sites of M13mp19 and pUC19 and sequenced by the Sanger dideoxy termination method[14] and by the Maxam and Gilbert chemical degradation method[15].

acids (Met-Leu-Trp-Trp-Glu-Glu-Val-Glu-Asp-Cys-Tyr) of the muscle protein are replaced by three amino acids (Met-Glu-Asp) in brain dystrophin.

As the second exon is identical in both mRNA types, brain mRNA should also protect a 32-base pair (bp) fragment of probe *A* (Fig. 1), but we were unable to detect such a fragment in our assays (Fig. 2). This might be due to the very low G+C content (28%) of this region of the probe. The 5′ half of the probe has a 45% G+C content, permitting stable hybridization when the entire probe is protected by muscle mRNA. As seen in Fig. 2, we can also detect DMD mRNA in spleen (5–10% of the amount in striated muscle), and smaller amounts are detected in the lung and testis. At the present level of sensitivity of the assay system, DMD mRNA was barely detectable in kidney and was not detectable in liver RNA preparations.

These results show that the DMD gene, which in myogenic cells is developmentally regulated, is also expressed in non-myogenic tissues. Detection of DMD mRNA and protein in non-muscle tissues has also been reported recently by Chelly *et al.*[12] and Hoffman *et al.*[13] respectively. We have found that the DMD gene is also expressed in cultured brain cells (in collaboration with R. Simantov, unpublished results). This observation and the finding that the brain DMD mRNA is not identical to that of the muscle clearly demonstrates that the DMD RNA found in the brain tissue originated from brain cells and not from the smooth muscle of blood vessels. Experiments are currently being carried out to identify the DMD isoforms and the cell types that express the DMD gene in other non-muscle tissues.

Genetic considerations and Southern blot analysis have indicated the existence of only one DMD gene[3,5] Two mechanisms could, separately or together, be responsible for the production of more than one mRNA from a single gene: (1) alternative splicing of an exon or exons from the gene transcript and (2) alternative initiation of transcription from different promoters reviewed in ref. 10). The finding that sequences of the 5′ end of the muscle and brain DMD mRNAs are different indicates that two promoters with different tissue and developmental specificities could be involved in the initiation of transcription to produce the different mRNAs.

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Effects of the tumour promoter okadaic acid on intracellular protein phosphorylation and metabolism

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Okadaic acid is a polyether derivative of 38-carbon fatty acid[1], and is implicated as the causative agent of diarrhetic shellfish poisoning[2]. It is a potent tumour promoter that is not an activator of protein kinase C (ref. 3), but is a powerful inhibitor of protein phosphatases-1 and -2A (PP1 and PP2A) *in vitro*[4,5]. We report here that okadaic acid rapidly stimulates protein phosphorylation in intact cells, and behaves like a specific protein phosphatase inhibitor in a variety of metabolic processes. Our results indicate that PP1 and PP2A are the dominant protein phosphatases acting on a wide range of phosphoproteins *in vivo*. We also find that okadaic acid mimics the effect of insulin on glucose transport in adipocytes, which suggests that this process is stimulated by a serine/threonine phosphorylation event.

The first insight into the mechanism of action of okadaic acid came from the finding that it enhanced the contraction of skinned smooth muscle fibres, indicating that myosin P-light chain phosphorylation was increased[6]. This appeared to be mediated by inhibition of protein phosphatase(s) as myosin light chain kinase activity was unaffected and a phosphatase from smooth muscle with activity towards isolated myosin P-light chains was inhibited by okadaic acid[7]. The toxin was recently shown to be a potent inhibitor of PP1 and PP2A (refs 4 and 5), which have homologous catalytic subunits[8] and make up two of the four cytosolic serine/threonine-specific protein phosphatase catalytic subunits in mammalian cells[9–11]. PP2B (a calcium/calmodulin-dependent enzyme) was inhibited with much lower potency, whereas PP2C (a magnesium-dependent enzyme) was unaffected[4,5].

Okadaic acid produces marked increases in the phosphorylation states of many proteins in adipocytes and hepatocytes, as judged by increased ^{32}P-labelling (Fig. 1), without changing the specific radioactivity of intracellular ATP or the ATP:ADP ratio (data not shown). Proteins whose phosphorylation increased included polypeptides that comigrated with acetyl-CoA carboxylase and ATP-citrate lyase in adipocyte and hepatocyte cytosol, pyruvate kinase and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK2/FBPase) in hepatocyte cytosol, and glycogen phosphorylase and glycogen synthase in the hepatocyte glycogen fraction. The prominent protein of relative molecular mass 32,000 in the hepatic microsomal fraction whose phosphorylation increased markedly in response to okadaic acid is likely to be ribosomal protein S6. Okadaic acid treatment (15 min) stimulated the ^{32}P-labelling of total trichloroacetic acid-insoluble protein in hepatocyte cytosol 2.5-fold, with a half-maximal effect at 200 nM and maximal effect at 1 μM.

The protein kinases which phosphorylate the above-mentioned proteins *in vivo* include cyclic AMP-dependent protein kinase[12], the AMP-activated protein kinase[13], phosphorylase kinase[14], glycogen synthase kinase-3 (ref. 15) and casein kinase-2 (refs 16 and 17). Using glycogen synthase as substrate, okadaic acid at concentrations as high as 5 μM failed to affect the activity of any of these protein kinases, or casein kinase-1 (data not shown). The purified catalytic subunits of PP1 and PP2A were potently inhibited by okadaic acid with 50% inhibitory concentration values of 20 nM and 0.2 nM respectively which are even lower than those reported previously[4,5]. The PP2A catalytic subunit was completely inhibited by 1 nM

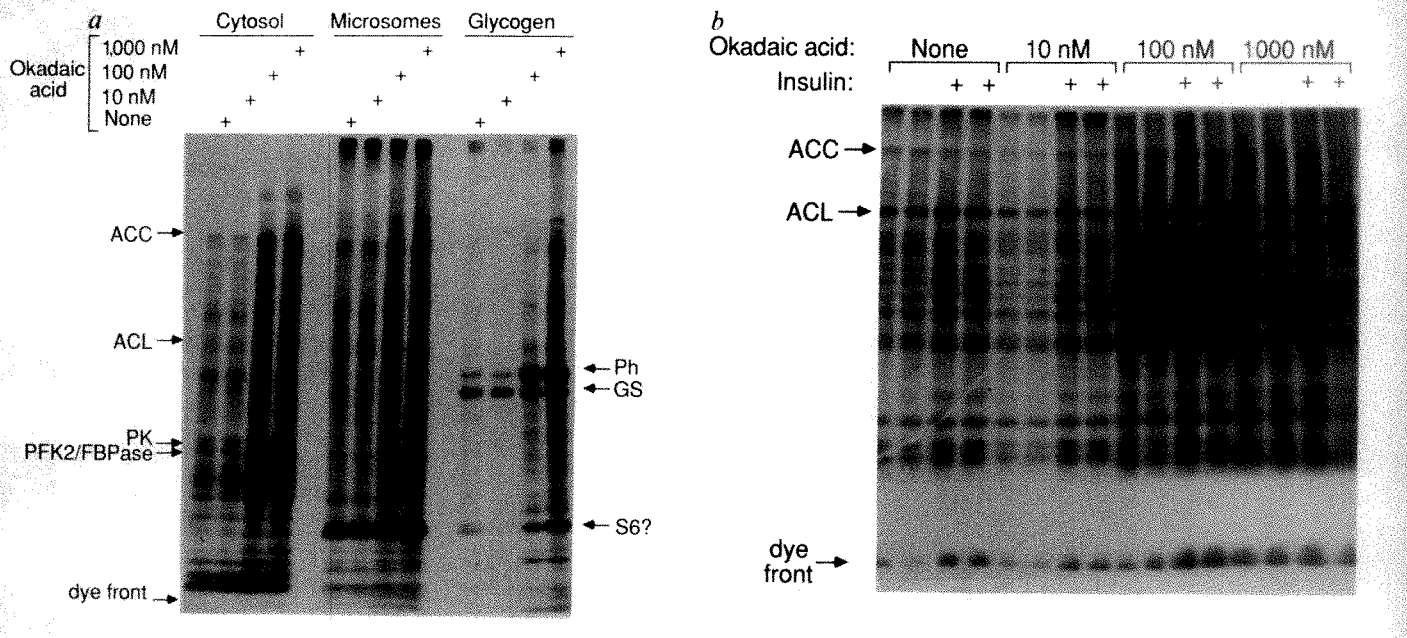


Fig. 1 Effects of okadaic acid on protein phosphorylation in isolated hepatocytes (*a*) and adipocytes (*b*) from fed rats. For adipocytes, incubations were carried out in duplicate in the presence and absence of 0.9 nM insulin. Cells were isolated and prelabelled with [^{32}P]phosphate for 60 min[20,21] before addition of okadaic acid or insulin for 15 min. Okadaic acid was added in 10% dimethylsulphoxide to give a final concentration of dimethylsulphoxide of 0.25% (v/v) in all incubations. Cells were collected and homogenized, and post-mitochondrial supernatants were prepared by centrifugation (10,000*g* for 15 min at 4 °C). For hepatocytes only, the post-mitochondrial supernatant was separated into cytosol, glycogen and microsomal fractions by centrifugation (100,000*g* for 60 min at 4 °C). The supernatants were removed (cytosol fraction), and the upper microsomal pellet was resuspended in homogenization buffer separately from the lower glycogen pellet. Fractions were analysed at equal protein loadings by electrophoresis in 5–15% gradient polyacrylamide gels. Gels were dried and autoradiographed at −70 °C using Amersham Hyperfilm-MP in Kodak X-omatic intensifying cassettes. The migration of marker proteins is shown, using the abbreviations: ACC, acetyl CoA carboxylase; ACL, ATP-citrate lyase; PK, pyruvate kinase; PFK2/FBPase, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; Ph, glycogen phosphorylase; GS, glycogen synthase; S6, ribosomal protein S6. The specific radioactivity of cellular ATP and ATP/ADP ratios were determined by HPLC (ref. 21).

okadaic acid, a concentration comparable with that of the phosphatase subunit in the assay.

As a number of the proteins in adipocytes and hepatocytes whose phosphorylation was increased by okadaic acid were enzymes of glucose and lipid metabolism, we examined the effects of okadaic acid on the flux through these pathways. Okadaic acid mimicked the action of glucagon in increasing the rate of glucose output from hepatocytes, as well as the conversion of lactate to glucose (Fig. 2), as would be expected if the toxin increased phosphorylation of glycogen phosphorylase, glycogen synthase, pyruvate kinase and PFK2/FBPase. Okadaic acid stimulated basal lipolysis in adipocytes 2.6-fold (Fig. 3*a*), which is consistent with increased phosphorylation of hormone-sensitive lipase. A larger stimulation of lipolysis (10-fold) was observed in the presence of the adenosine agonist PIA, which causes inhibition of adenylate cyclase and reduces basal cAMP-dependent protein kinase activity and lipolysis[17] (Fig. 3*c*). Okadaic acid also completely prevented insulin from antagonizing the β-adrenergic activation of lipolysis (Fig. 3*b*).

Phosphorylation is known to inhibit acetyl CoA carboxylase, the rate-limiting enzyme in fatty acid biosynthesis[18], and we found that the activity of this enzyme was greatly decreased in extracts from okadaic acid-treated adipocytes (Fig. 3*d*). Acetyl CoA carboxylase activity itself was unaffected by inclusion of okadaic acid (1 μM) in the assays. There was a good correlation between inactivation of acetyl-CoA carboxylase and inhibition of insulin-stimulated fatty acid synthesis, as measured by incorporation of radioactivity from ^{14}C-labelled acetate into total lipid (Fig. 3*d*). Okadaic acid also inhibited the low rate of fatty acid synthesis observed in the absence of insulin (not shown).

Although okadaic acid antagonized the actions of insulin on fatty acid synthesis and lipolysis (Fig. 3), it mimicked the action

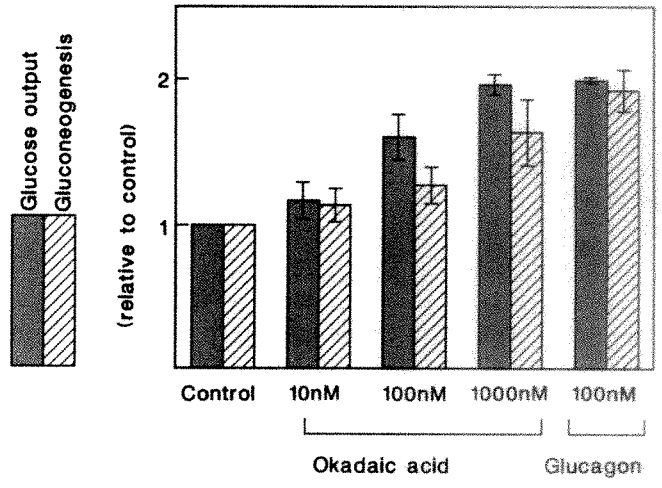


Fig. 2 Effects of okadaic acid on glucose output and gluconeogenesis in isolated hepatocytes from fed rats. Hepatocytes were isolated in medium containing 10 mM glucose and preincubated for 10 min with okadaic acid as in Fig. 1, except that the medium contained unlabelled phosphate. Gluconeogenesis was measured by the incorporation of radioactivity from [^{14}C]lactate into glucose[23]. Glucose output was measured using glucose oxidase (Sigma diagnostic kit 510-A); for these studies only, cells were collected by centrifugation and resuspended in medium without glucose immediately before addition of okadaic acid.

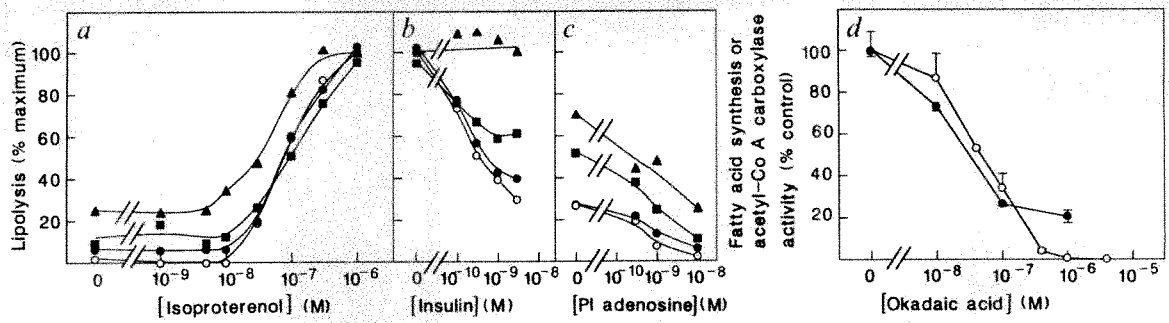
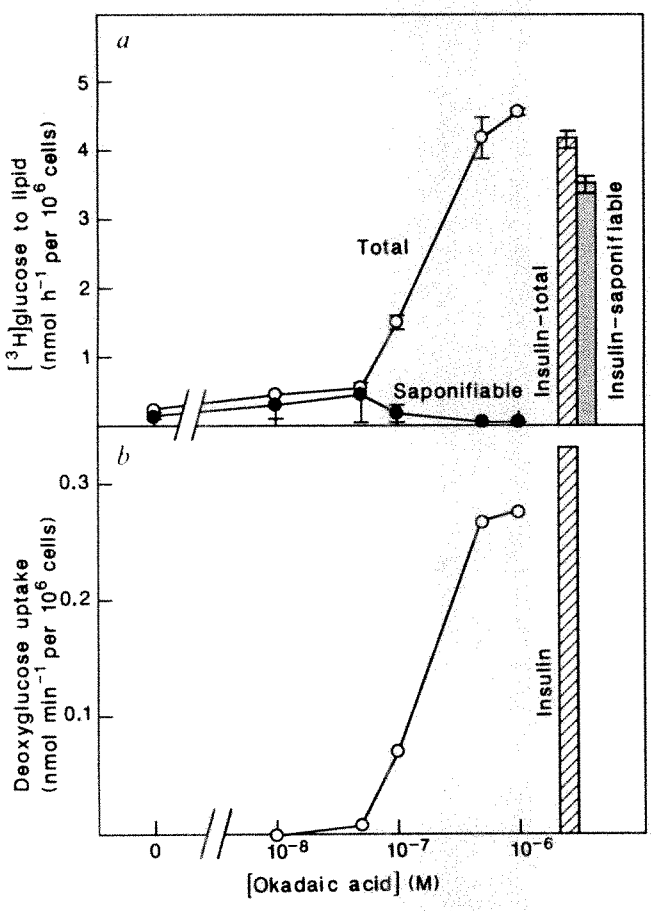


Fig. 3 Effects of okadaic acid treatment on metabolic parameters of isolated adipocytes. *a–c*, Effects of incubation for 25 min without okadaic acid (open circles), or with okadaic acid at 100 nM (filled circles), 300 nM (squares) or 1 μM (triangles) on lipolysis measured as glycerol release[17]. The adenosine agonist PIA (N^6-[*R*-(−)-1-methyl-2-phenethyl]adenosine, 100 nM) was present in *a*, and isoproterenol (1 μM) and PIA (100 nM) were present in *b*. *d*, Incorporation of radioactivity from [^{14}C]acetate into toluene extractable-lipid[24] (open circles) and acetyl CoA carboxylase activity (closed circles) measured at 10 mM citrate in the post-mitochondrial supernatant fraction[20].



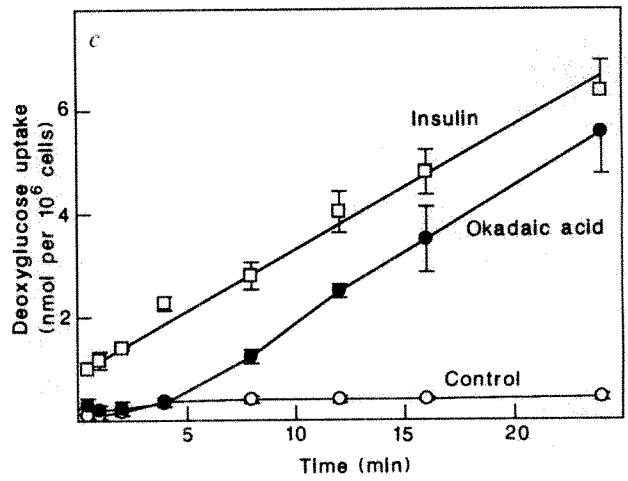


Fig. 4 Effects of okadaic acid and insulin on conversion of glucose into lipid and on 2-deoxyglucose uptake in adipocytes. *a*, Effect of okadaic acid on incorporation of radioactivity from [^{3}H]glucose into total toluene-extractable lipid (open circles) or saponifiable lipid (filled circles)[20]. The effects of a 10 min incubation with 0.9 nM insulin (total lipid, cross-hatched bar; saponifiable lipid, shaded bar) are also shown for comparison. *b*, Effect of okadaic acid on uptake of [^{14}C]-labelled 2-deoxyglucose into cells. The effect of a 10 min incubation with 0.9 nM insulin is shown by the cross-hatched bar. For both *a* and *b*, cells were preincubated with okadaic acid for 10 min before the measurement. *c*, Time course for uptake of [^{14}C]-labelled 2-deoxyglucose into cells treated in the absence of okadaic acid or insulin (open circles), or in the presence of 0.9 nM insulin (open squares) or 1 μM okadaic acid (filled circles). Deoxyglucose uptake was measured by incubating cells with 0.5 mM [U-^{14}C] 2-deoxyglucose (0.2 μCi ml^{-1}). Reactions were stopped by adding 200 μl aliquots of cells to 50 μM cytochalasin B (50 μl) and 50 μM phloretin in 0.25 × 4 cm polythene microtubes. 100 μl silicone oil (0.97 g ml^{-1}) was layered on top of the cells, and the tubes were then spun at 7,000*g* for 5 s in a bench centrifuge. The top half of the tubes containing the cells was cut off with a scalpel and dropped into 1 ml scintillation fluid (LKB Optiphase II) for counting. Control incubations showed that cytochalasin B and phloretin completely inhibited 2-deoxyglucose uptake in response to okadaic acid.

of insulin in dramatically enhancing the incorporation of radioactivity from [^{3}H]glucose into total lipid (Fig. 4*a*). But whereas 84% of the radioactivity incorporated into lipid was saponifiable (as fatty acid) in the case of insulin, <5% was saponifiable after treatment with okadaic acid. The simplest explanation for these results is that okadaic acid stimulated glucose transport while activating lipolysis and inhibiting fatty acid synthesis, so that most of the increased radioactivity would be glycerol-incorporated during triglyceride turnover. Insulin, however, stimulates glucose transport while inhibiting lipolysis and activating fatty acid synthesis, and radioactivity incorporated from glucose into triglyceride in this case would largely be present in fatty acid. To measure the effect of okadaic acid on glucose transport more directly, we studied the uptake of 2-deoxyglucose into adipocytes. After a lag of about 5 min, okadaic acid greatly stimulated uptake. Insulin produced the same effect, but without a significant lag (Fig. 4).

Our results strongly support the view that the effects of okadaic acid on cell function are explained by its ability to inhibit PP1 and/or PP2A. All effects on intact cells were maximal at 1 μM, which is similar to the *in vivo* concentration of PP1 and PP2A (ref. 11). None of the eight protein kinases tested to date (our data and refs 3 and 7) were affected by okadaic acid. Okadaic acid at 1 μM scarcely affects PP2B activity[5], and its specificity

is further emphasized by its failure to inhibit PP2C (ref. 4), pyruvate dehydrogenase phosphatase (T.A.J.H., unpublished results), protein tyrosine phosphatases, acid and alkaline phosphatases, and inositol trisphosphatase[4]. Furthermore, because okadaic acid stimulates glycogenolysis and gluconeogenesis in hepatocytes, and lipolysis and the conversion of glucose to glyceride-glycerol in adipocytes (Figs 2–4), and does not affect ATP or ADP levels, none of the enzymes in these metabolic pathways appears to be inhibited non-specifically by this compound.

Assays in cell-free extracts have shown that PP1, PP2A and PP2C account for virtually all the phosphatase activity in skeletal muscle and liver towards some 20 phosphoproteins that regulate several metabolic pathways and muscle contraction[9–11]. This study now strongly suggests that PP1 and PP2A, rather than PP2C, are the dominant phosphatase catalytic subunits acting on a wide range of phosphoproteins *in vivo*. The toxin is therefore a powerful probe for the investigation of the role of protein phosphorylation and of PP1/PP2A in any cellular event. It has already been shown to increase the calcium current in isolated myocytes[5], suggesting that the slow inward calcium channel is activated by phosphorylation in cardiac cells, and inactivated by PP1 and/or PP2A.

Although most of the effects of okadaic acid on protein phosphorylation and metabolism could have been predicted, the stimulation of glucose transport was unexpected, and the results are evidence that this process is activated by a protein serine/threonine phosphorylation event. The failure to observe phosphorylation of the glucose transporter in response to insulin[19], suggests that an associated regulatory protein may be the target for phosphorylation, rather than the transporter itself.

Because PP1 and PP2A are likely to be the chief enzymes that reverse the actions of protein kinase C, it is not surprising that a phosphatase inhibitor which can enter intact cells should be as potent a tumour promoter as substances that activate protein kinase C. Tumour promotion presumably stems from increased phosphorylation of one or more proteins that are substrates for protein kinase C and dephosphorylated by PP1/PP2A. The effects of okadaic acid on protein phosphorylation in isolated cells (Fig. 1) emphasize what a powerful tumour-suppressing effect PP1 and PP2A must have on normal cells.

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The pseudoautosomal boundary in man is defined by an *Alu* repeat sequence inserted on the Y chromosome

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The Y chromosome, which in man determines the male sex (reviewed in ref. 1), is composed of two functionally distinct regions. The pseudoautosomal region is shared between the X and Y chromosome and is probably required for the correct segregation of the sex chromosomes during male meiosis[2]. The second region includes the sex-determining gene(s), the presence of which is necessary for the development of testes. The two regions have contrasting genetic properties: the pseudoautosomal region recombines between the X and Y chromosome[3]; the Y-specific region must avoid recombination otherwise the chromosomal basis of sex-determination breaks down[1]. The pseudoautosomal region is bounded at the distal end by the telomere and at the proximal end by X- and Y-specific DNA. We have found that the proximal boundary was formed by the insertion of an *Alu* sequence on the Y chromosome early in the primate lineage. Proximal to the *Alu* insertion there is a small region where similarity between the X and Y chromosomes is reduced and which is no longer subject to recombination.

Previous studies provided us with a defined region for the location of the pseudoautosomal boundary[4]. The *MIC2* gene is the closest marker known distal to the pseudoautosomal boundary and the probe (3′Rsa) is derived from the 3′-most exon of the *MIC2* gene. *DYS104* is the most distal Y-specific sequence that has been described[4]. To isolate the pseudoautosomal boundary we cloned 110 kilobases (kb) of DNA between the sequences defined by 3′Rsa and *DYS104* (Fig. 1). The Y-derived cosmid cAMF31 contains sequences that cross the pseudoautosomal boundary because it includes both Y-specific (H2.1) and pseudoautosomal sequences (E1.8). This places the boundary between 30 kb and 45 kb away from the sequences defined by 3′Rsa.

Two probes (Hf0.2 and RsY0.55) from cosmid cAMF31 were used to define the pseudoautosomal boundary: both probes are located on a Y-specific *Sac*I fragment of 3.3 kb (SacIY3.3; Fig. 2*a*). Hybridization of Hf0.2 to *Eco*RI and *Hae*III digests of DNA from females, from males and from somatic cell hybrids having the X chromosome and the Y chromosome as their only human contribution, reveals fragments of 1.8 kb and of 0.6 kb respectively (Fig. 2*b*, lanes 1–8). When Hf0.2 is hybridized to the same DNAs after *Sac*I digestion, fragments of 3.3 kb and 4.5 kb appear in male DNA but only one fragment of 4.5 kb is evident in female DNA (Fig. 2*b*, lanes 13 and 14). The 3.3-kb fragment is specific to the Y chromosome (lane 16) and the 4.5-kb fragment is specific to the X chromosome (lane 15). Similarly, Hf0.2 recognizes 2.4-kb and 2.8-kb *Rsa*I fragments in male DNA and only a 2.8-kb fragment in female DNA (lanes 9 and 10). The 2.4-kb fragment comes from the Y (lane 12), and the 2.8-kb fragment comes from the X (lane 11). Hence, the pseudoautosomal Hf0.2 probe maps Y-specific and X-specific restriction sites. The pseudoautosomal boundary lies between the *Eco*RI and *Rsa*I sites proximal to the sequences defined by Hf0.2.

The results in Fig. 2*c* confirm this conclusion. Hybridization of the RsY0.55 probe to the *Sac*I-digested DNAs presented in Fig. 2*b* reveals the 3.3-kb fragment in male and 'Y-only' DNA, but not in female and 'X-only' DNA. DNA from 64 females

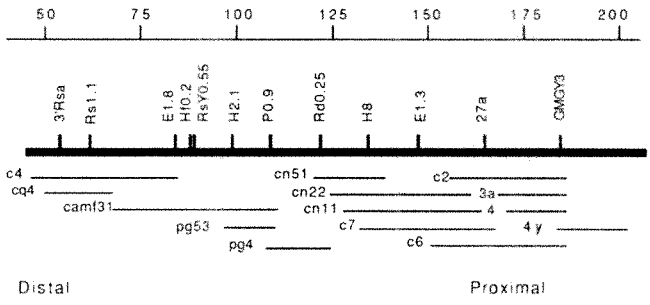


Fig. 1 Map of the region cloned in the chromosome walk. If the 5' CpG-rich region of *MIC2* is 0 kb, we set 3'Rsa at 55 kb on the long-range restriction map on the basis of partial cloning and Southern blotting of the *MIC2* gene[4,8]. Unique probes were isolated at each stage of the walk and their chromosomal origin determined by hybridization to gel transfers of DNA from female (WT49), male (PGF), 'X-only' somatic cell hybrid (Cl2D) and 'Y-only' somatic cell hybrid (853). Probe designations and clone sizes are shown. Cosmid clones are preceded by 'c'; all others are lambda clones. Probe 27a defines *DYS104* and GMGY3 defines *DYS13*. Probes H8, Rd0.25 and E1.8 need competition with sheared human placenta DNA[9].

Methods. Three cosmid libraries (one was donated by Peter Little and constructed with male DNA; the two others were constructed with male DNA and 853 DNA) and four lambda libraries (HL100 was constructed with male DNA and purchased from Clontech, and the other three were constructed with 853 DNA) were used in screening. Libraries were made by ligating genomic DNA, either partially digested with *Mbo*I or completely digested with *Eco*RI or *Hin*dIII, into appropriately digested pcos8 or λDASH (Stratagene) DNA, followed by packaging and transduction into P2392, ED8767 or NM621 *Escherichia coli* strains. Hybridization was in 6×SSC, 5×Denhardt's solution, 0.5% SDS, 0.25 mg ml^{-1} sheared and denatured DNA from salmon testes, and 2 ng ml^{-1} labelled probe. Probes were isolated on NA45 paper and labelled with ^{32}P by oligonucleotide reaction[10]. For hybridization with cosmid libraries, 0.1 mg ml^{-1} *Hae*III-digested and denatured vector DNA was added to prevent non-specific binding. The filters were washed twice at room temperature in 2×SSC and 0.2% SDS, then 3–5 times for 15 min in 0.2×SSC and 0.2% SDS at 65 °C, mounted and then exposed to XAR-5 with Cronex lightning-plus intensifier screens overnight.

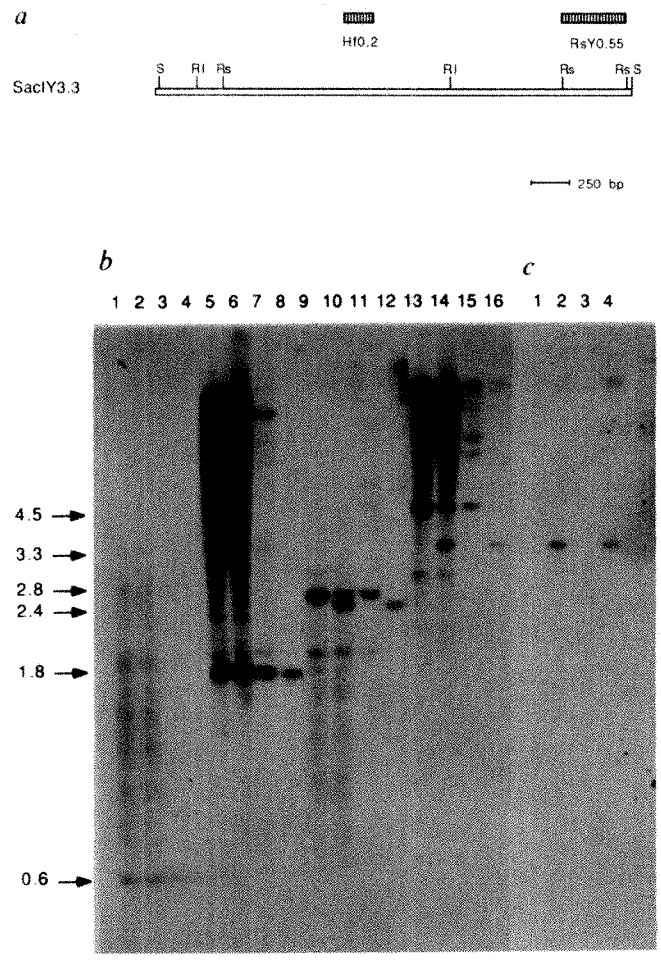


Fig. 2 Mapping the pseudoautosomal boundary with the Hf0.2 probe. *a*, Restriction map of SacIY3.3 and the two probes derived from it. Hf0.2 defines pseudoautosomal sequences and RsY0.55 defines Y-specific sequences. The 3.3-kb *Sac*I fragment from cAMF31 was subcloned into the *Sma*I site of pUC8. Hf0.2 and RsY0.55 were also subcloned into the *Sma*I site of pUC8. S, *Sac*I; RI, *Eco*RI; Rs, *Rsa*I. *b*, The Hf0.2 probe was hybridized to female, male, 'X-only' hybrid and 'Y-only' hybrid DNAs: WT49 (lanes 1, 5, 9 and 13), PGF (lanes 2, 6, 10 and 14), C12D (lanes 3, 7, 11 and 15) and 853 (lanes 4, 8, 12 and 16). Lanes 1–4 show DNA digested with *Hae*III; lanes 5–8, with *Eco*RI; lanes 9–12, with *Rsa*I and lanes 13–16, with *Sac*I. *c*, RsY0.55 was hybridized to the filter used in *b* after stripping it first. Lanes 1–4 are *Sac*I-digested DNA from female, male, 'X-only' hybrid and 'Y-only' hybrid.

Methods. Each DNA (10 μg) was digested according to the supplier's instructions, electrophoresed through a 0.7% agarose gel and transferred on to GeneScreen Plus (NEN). Hybridization, post-hybridization washing and autoradiography have been previously described[11].

and 57 males was hybridized with the RsY0.55 probe: all the male samples, but none of the female samples, reacted with the probe. Therefore, the proximal pseudoautosomal region rarely, if ever, includes the sequences defined by RsY0.55 located on the Y chromosome.

X-specific sequences adjacent to the boundary were isolated by screening a lambda library with a probe derived from SacIY3.3. Overlapping clones λX31 and λX21 contain the pseudoautosomal 1.8-kb *Eco*RI fragment and the X-specific 4.5-kb *Sac*I fragment. The 4.5-kb *Sac*I fragment (SacIX4.5), which contains the X-side of the pseudoautosomal boundary, was subcloned and mapped using restriction enzymes (Fig. 3). The maps of SacIX4.5 and SacIY3.3 are nearly identical as far as the *Pst*I sites in the middle of each clone, but beyond this they are dissimilar. The pseudoautosomal boundary seems to lie in the region of ~50-base pair (bp) between the *Msp*I and *Hha*I sites on the Y chromosome (Fig. 3).

The 0.9-kb *Eco*RI/*Rsa*I fragment from SacIY3.3 (RsE0.9) and the 0.8-kb *Pst*I fragment from SacIX4.5 (P0.8) were subcloned (Fig. 3) and sequenced (Fig. 4*a* and *b*). As predicted by the restriction map, the boundary is located near the *Hha*I site. The two sequences diverge dramatically 245 bp proximal to the pseudoautosomal *Eco*RI site. Only two bases differ between the Y and X chromosomes at locations 201 bp and 205 bp from the *Eco*RI site. At the boundary on the Y chromosome, a 303-bp *Alu* repeat is inserted (Fig. 4*b*) and the first base pair of the *Alu* insertion is the first base-pair difference between the X and Y chromosomes. On the other side of the *Alu* insertion, homology between the X and Y chromosomes resumes and extends for 225 bp. The X and Y show 77% homology in this region. Beyond this point the sequences cannot be aligned by the IFIND:ALIGN (Intelligenetics) program. Figure 4*c* shows a schematic representation of the boundary region. Two regions of homology are separated by an *Alu* insertion, and proximal to these the sequences are non-homologous.

We propose that the pseudoautosomal boundary is precisely located at the point where the *Alu* sequence is inserted on the Y chromosome. Recombination has prevented sequence divergence in the region distal to the *Alu* (*Alu*-distal region). If the

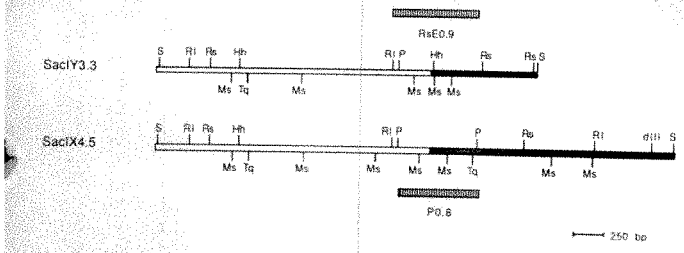
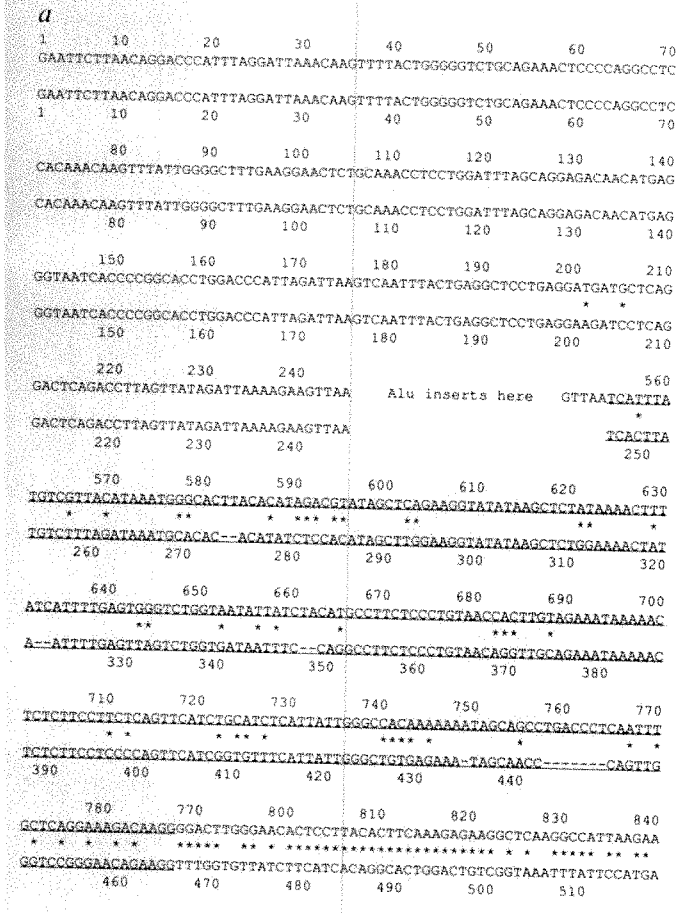


Fig. 3 A restriction map of the pseudoautosomal boundary. The lambda clones from which the X-specific 4.5-kb *Sac*I fragment is derived were isolated from a flow-sorted X-chromosome library (Los Alamos, ID code LA0XNL01). The fragment was subcloned into the *Sac*I site of Bluescript[c] (Stratagene). RsE0.9 was subcloned into *EcoR*I/*Sma*I-digested M13mp18 DNA and P0.8 into *Pst*I-digested pUC8 DNA. One *Msp*I site present in the SacIX4.5 is missing in the SacIY3.3. This site is associated with a restriction-fragment length polymorphism (data not shown).



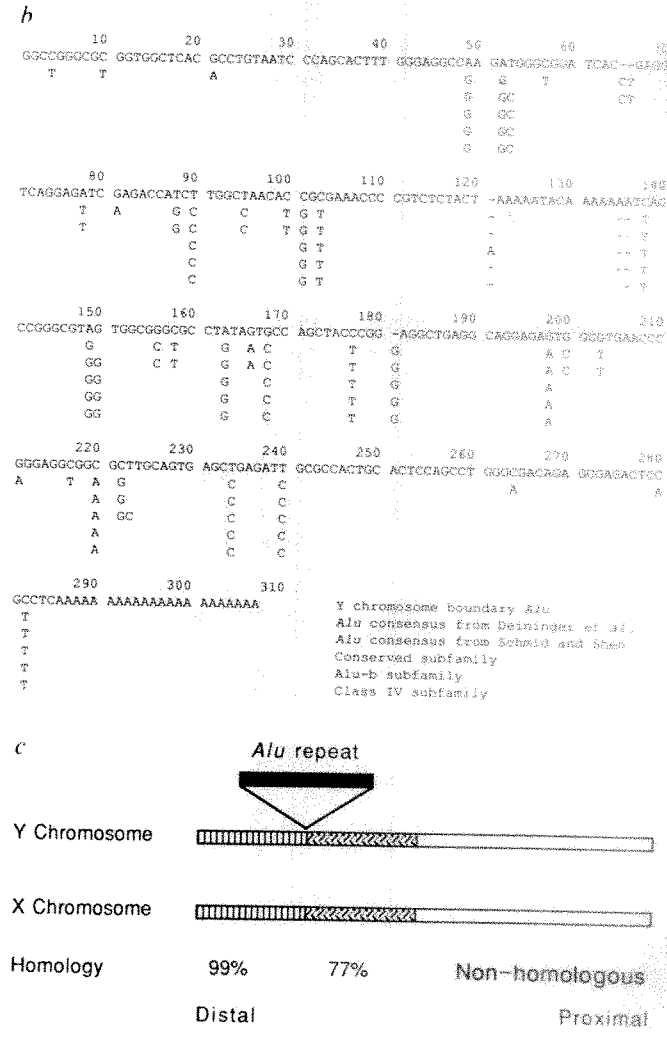


Fig. 4 DNA sequence of the pseudoautosomal boundary. Subclones were derived from the RsE0.9 and P0.8 in either pUC8 or Bluescript[c] and sequenced in double-stranded DNA by the dideoxy chain-termination method[12,13]. Oligonucleotides were used to complete the project. The sequence from the *Eco*RI site to the distal *Pst*I site on the X chromosome was derived from the appropriate 1.8-kb *Eco*RI subclone of SacIX4.5. *a*, The DNA sequence before and following the *Alu* insert on the X and Y chromosome is compared by removing the *Alu*. The *Alu*-proximal region is shown underlined, mismatches are starred and gaps are dashed. Note that multiple base changes frequently occur in the *Alu*-proximal region and that the gaps are all on the X chromosome. The complete sequence of the 947-bp RsE0.9 fragment and 771 bp from *Eco*RI to *Pst*I from SacIX4.5 is available on request. *b*, The *Alu* insert is compared with the consensus sequences from Deininger *et al.*[14] and from Schmid and Shen[15], and with the subfamily consensus sequences from Willard *et al.*[16], Jurka and Smith[17], and Britten *et al.*[18]. The *Alu* insertion appears to be a retroposition[19] because it ends with a string of 22 adenine nucleotides and has 5-bp direct repeats at each end (GTTAA). The *Alu* belongs to the conserved subfamily and has diverged by 6–7% from this group. *c*, Schematic drawing of the pseudoautosomal boundary shows the aligned *Alu* proximal and *Alu* distral regions with percentage homology represented as hatched lines, the *Alu* insert as a solid line, and X- and Y-specific regions as open lines.

mutation rate exceeded the probability of crossover between the mutation and the boundary, mutations would accumulate near the boundary. The pseudoautosomal region undergoes recombination at an average rate of 1% (10^{-2}) per 5×10^4 bp, therefore the recombination rate is 2×10^{-7} per meiosis per base pair[3]. If we make the parsimonious assumption that there is one meiosis per generation, and three generations every 100 years, we get a recombination rate of 6×10^{-9} per bp per year. Given the rate of sequence evolution is $\sim1.5\times10^{-9}$ mutations per site per year[5], then the rate of recombination slightly exceeds the mutation rate at the boundary. The *Alu*-distal region shows only two base differences out of 245 bp, which is consistent with our analysis; these two differences may be pseudoautosomal polymorphisms.

The fact that the region proximal to the *Alu* (*Alu*-proximal region) has diverged 23% implies that recombination has not maintained sequence similarity in this region. If recombination

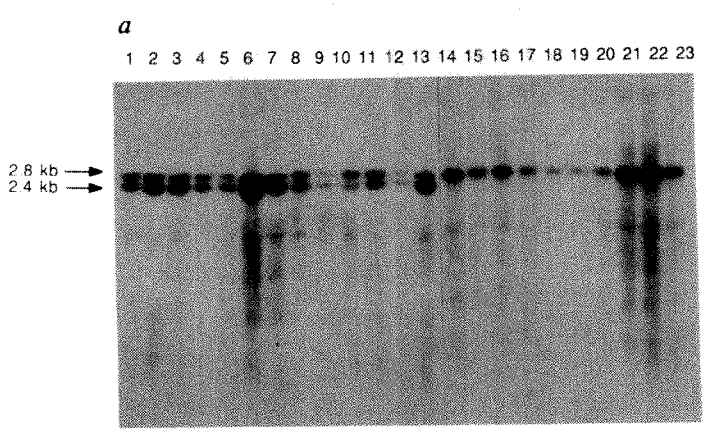


Fig. 5 Hybridization of the Hf0.2 probe to *Rsa*I-digested DNA from males and females. Female DNA shows a single 2.8-kb X-derived fragment; male DNA gives a 2.8-kb X-derived fragment and a 2.4-kb Y-derived fragment. *a*, DNAs from human males are in lanes 1–13 and DNAs from females in lanes 14–23. A total of 50 males, 25 females, an 'X-only' and a 'Y-only' hybrid was tested. Gel transfers were hybridized with RsY0.55 to test for Y-specific DNA. *b*, Male (odd-numbered lanes) and female (even-numbered lanes) DNAs from human (lanes 1 and 2), chimpanzee (lanes 3 and 4) and gorilla (lanes 5 and 6).

were occurring in the *Alu*-proximal region, then the *Alu* insertion would be present in females as well as in males. To test this hypothesis, we hybridized the Hf0.2 probe to *Rsa*I-digested DNA from males and females. Hf0.2 detects *Rsa*I fragments of 2.4 kb and 2.8 kb which cross the pseudoautosomal boundary. If recombination is occurring within the *Alu*-proximal region, the *Alu* is transferred to the X and increases the size of the X-specific *Rsa*I fragment to 3.1 kb, decreasing the Y-specific *Rsa*I fragment to 2.1 kb. We have already shown that sequences defined by the RsY0.55 are not found in females, but if recombination were to occur within or proximal to this locus, then hybridization with Hf0.2 would reveal a 2.4-kb *Rsa*I fragment in female DNA. We have tested 50 males and 25 females for this effect (see Fig. 5*a* for a subset of these results). In all cases, males had two fragments of 2.4 kb and 2.8 kb and females had only a 2.8-kb fragment. Moreover, in DNA from male and female gorillas and chimpanzees, we detected *Rsa*I fragments of identical size (Fig. 5*b*). We conclude that *Alu* insertion is of ancient origin and that recombination is not occurring in the *Alu*-proximal region. The base differences in the *Alu*-proximal region are uniformly distributed throughout the 225 bp, which suggests that divergence began for the whole region at once. This allows us to estimate the age of the human pseudoautosomal boundary. The common ancestor of the 225-bp region on the X and Y chromosome differs by 11.5% (23% ÷ 2) from that found today, which dates the boundary at ~45 million years old[6]. Unless other structural rearrangements have occurred, the previous boundary was 225 bp proximal to the *Alu* insertion and was also abrupt (Fig. 4*a*), as sequences proximal to the *Alu*-proximal region are non-homologous.

It is clear that the boundary has been created by the juxtaposition of nonhomologous sequences. Local maintenance of the boundary appears to be due, at least in part, to suppression of recombination caused by the nonhomologous sequences. Previously we suggested that the boundary could be limited by sex chromosome-specific functional genes or regulatory elements[7]. DNA sequencing and searching of databases has failed to provide evidence for such sequences close to the boundary on the X or the Y chromosome.

In summary, we have cloned and sequenced the pseudoautosomal boundary in man. The present-day boundary is defined by the insertion of a 303-bp *Alu* repeat sequence in the Y chromosome. Sequences immediately adjacent to the *Alu* insertion are identical, implying recombination occurs up to the *Alu*. The *Alu* insertion itself is Y-specific. Therefore the boundary is an abrupt change located at the *Alu*. Proximal to the *Alu* is a 225-bp region of X–Y homology which has diverged by 23%, suggesting the existence of an earlier boundary located 225 bp proximal to the *Alu*. We suggest that the absence of sequence homology was enough to create the pseudoautosomal boundary. Studies in primates should definitively date the *Alu* insertion.

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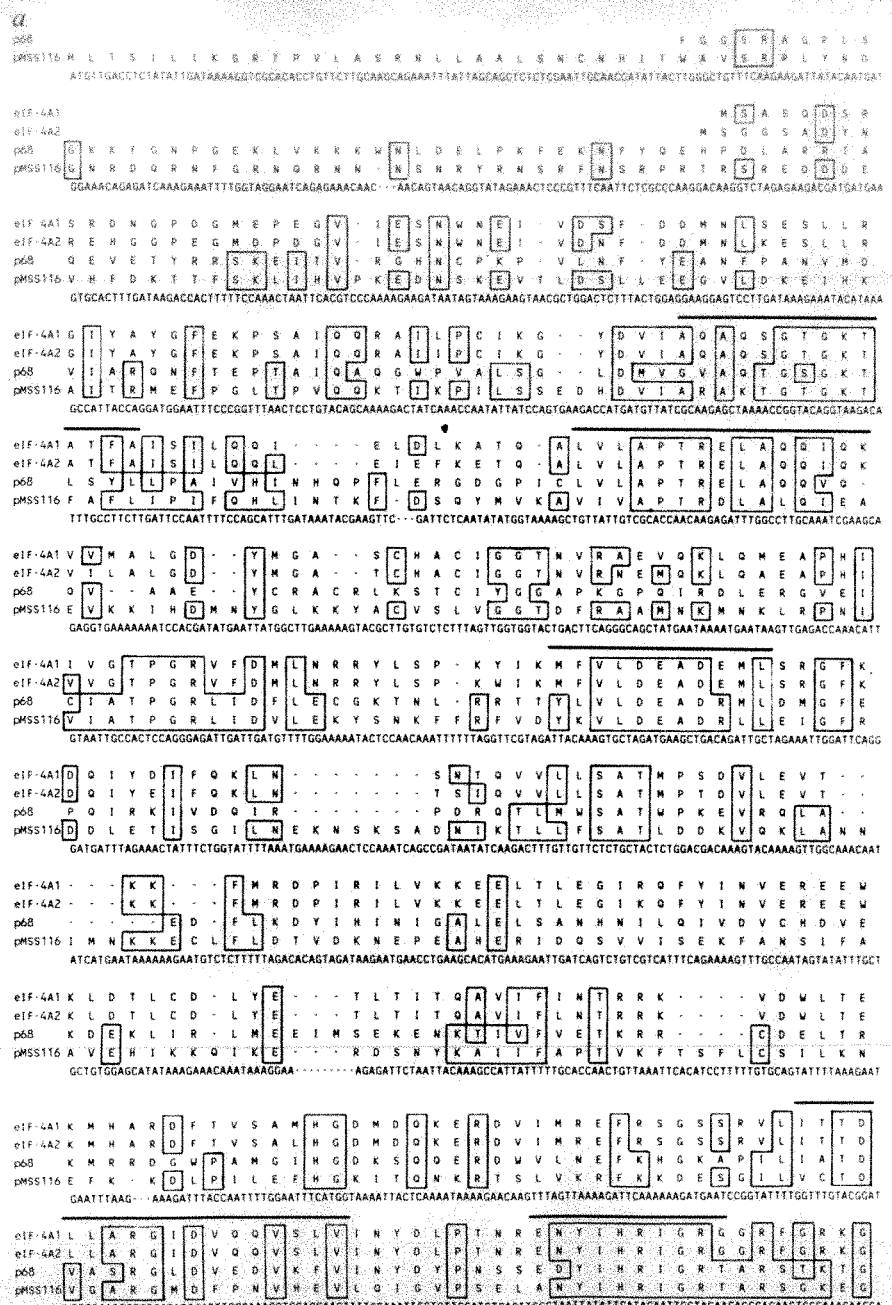
Mitochondrial splicing requires a protein from a novel helicase family

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Proteins involved in mitochondrial splicing but encoded by nuclear genes have been characterized in *Saccharomyces* and *Neurospora*[1–5]. The role in splicing of these proteins is largely unknown. Here we report that mutations in the nuclear gene *MSS116* directly affect the splicing of several introns of the cytochrome *b* (*cob*) and cytochrome *c* oxidase subunit I (*cox1*) primary transcripts. This implies that the MSS116 protein (pMSS116) is an important component of the mitochondrial splicing machinery. The sequence of the cloned *MSS116* gene shows that its protein product is homologous to the translation eIF-4A factor and the human nuclear protein p68. We show further that these proteins share several conserved amino-acid blocks with DNA helicases and related proteins. This suggests that pMSS116 has an RNA helicase activity. RNA helicases may be involved in many different processes including translation and splicing.

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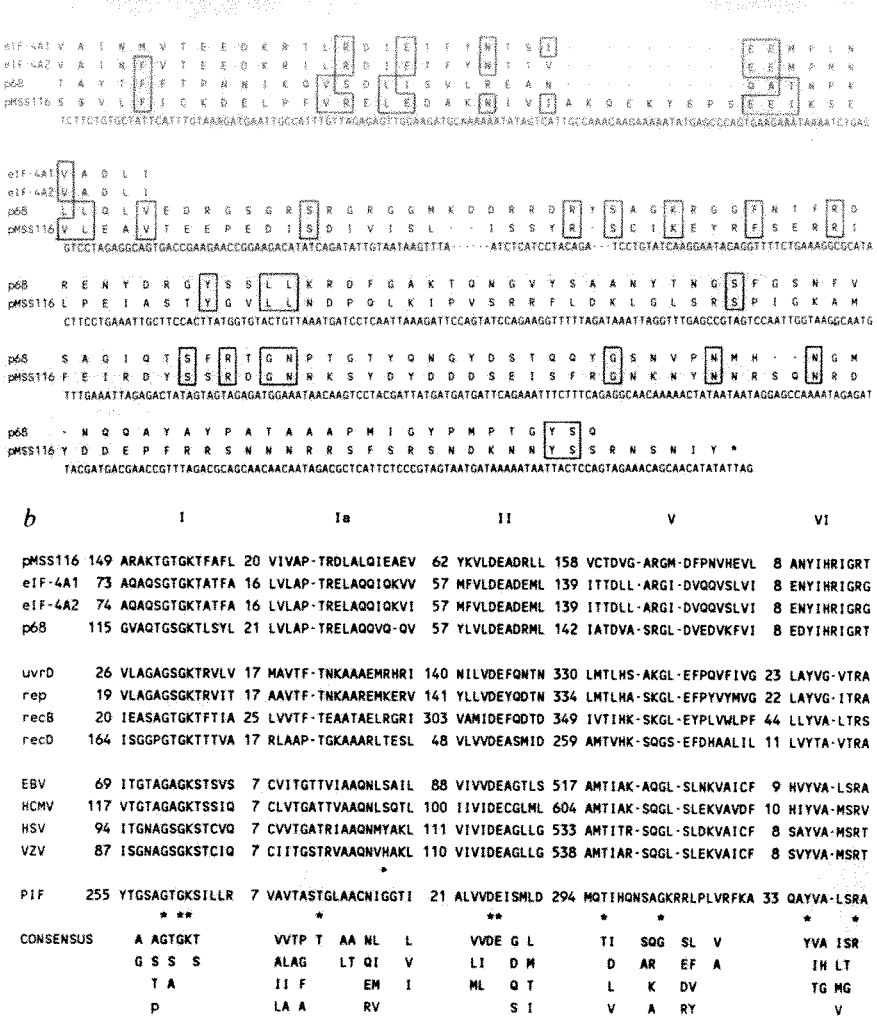


Fig. 1 Relationship of pMSS116 with eIF-4A, p68 and other helicases and replicative proteins. *a*, Alignment of the deduced amino-acid sequence of pMSS116 with the amino-acid sequence of eIF-4A1, eIF-4A2 (ref. 13) and p68 (ref. 14). Amino acids identical between pMSS116 and the other proteins are boxed. Note that conservative substitutions also occur at numerous positions. A hyphen indicates a gap introduced in the sequence to maximize similarity. Blocks I, I*a*, II, V and VI presented in *b* are overlined. The sequence of the *MSS116* gene is presented below the deduced pMSS116 protein sequence. *b*, Similarity between the eIF-4A/pMSS116/p68 protein family and helicases and replicative proteins. Blocks I, I*a*, II, V and VI have been defined by Hodgman for uvrD, rep, recB, recD, EBV, HCMV, HSV, VZV and PIF (see ref. 18 and refs therein). We have aligned for comparison the homologous blocks found in pMSS116, eIF-4A and p68. Blocks I and II probably form the ATP-binding site. Blocks Ia, V and VI have only been observed in helicase and replicative proteins but their function is unknown. Asterisks indicate amino acids conserved in all sequences. The consensus was derived by selecting positions where four or fewer amino acids occur.

We recently described a genetic screen for yeast nuclear mutants which specifically affect mitochondrial splicing[6]. Among the selected mutants, petA179 (renamed *mss116-2* here) is allelic to *mss116-1* which was shown, by Northern screening analysis, to impede the splicing of *cox1* transcript[7]. Northern experiments showed that mutation *mss116-2* has a drastic effect on the processing of primary transcripts (pre-mRNA) in a strain harbouring five introns in the *cob* gene and seven in the *cox1* gene (see Fig. 2 in ref. 6). A block in the splicing of one or several mitochondrial introns gives rise to a respiratory deficient phenotype. To find out which of the 13 known introns is dependent upon a wild-type MSS116 protein for splicing, mitochondrial genomes containing various sets of introns were introduced by cytoduction[6,8] into strains carrying either of the two mutant alleles. Lack of growth of the resulting cytoductants on glycerol (that is, their inability to respire) indicates which intron(s) requires the wild-type *MSS116* gene product for splicing. The results of these experiments, described in Table 1, indicate that excision of introns a1 as well as a1$5\alpha$ and/or a1$5\beta$ of *cox1* and b1, b2 and/or b3 of *cob* pre-mRNA depends upon the activity of pMSS116.

To gain further insight into the *MSS116* gene function, we cloned the wild-type allele by complementation in yeast and localized it within a 3-kb *Hin*dIII fragment. Its sequence shows a long reading frame coding for a putative protein of 664 amino acids (Fig. 1*a*). Gene disruption experiments[9] confirmed that we had cloned the *MSS116* gene (data not shown). Interestingly, introduction of mitochondria devoid of the 13 mitochondrial introns into a strain carrying a disrupted *MSS116* gene (allele *mss116-3*) does not restore respiratory competence. This suggests that pMSS116 has multiple functions. An alternative explanation is that some unidentified intron may exist in yeast mitochondria.

When we searched data bases for proteins homologous to the pMSS116 amino-acid sequence, we found a remarkable homology with the murine translation factor eIF4A (ref. 10) (Fig. 1*a*). To show that pMSS116 is directly involved in the splicing of *cob* and *cox1* pre-mRNA, and is not a translation initiation factor, we analysed the incorporation of [^{35}S]methionine into mitochondrial proteins *in vivo*[11]. Results obtained from strains bearing a disrupted *MSS116* gene are shown in Fig. 2. It appears that in a strain carrying the 13 known mitochondrial introns (Fig. 2, lane 1) the cob and cox1 proteins are absent, whereas the other major mitochondrial proteins are expressed. But we have not shown whether both the cox3 and ATPase 6 proteins are synthesized. When these mitochondria are exchanged by cytoduction for those of GF134-6D (see Table 1), protein cob is indeed translated and a low, though detectable, level of protein cox1 is synthesized (Fig. 2, lane 3). These results show that pMS116 is not a factor affecting general translation initiation *per se*. But our preliminary analyses of mitochondrial translation in mss116 strain does not rule out the possibility that pMSS116 is an initiation factor specifically required for efficient translation of the cox1, cox3 or ATPase 6 mRNA (Fig. 2, lane 3). An alternative possibility is that pMSS116 may be in some way necessary for the synthesis of some intron-encoded proteins (maturases). Northern experiments revealed that in the mitochondrial M12-54 context the splicing efficiency of intron b11 is greatly reduced by the *mss116-2* mutation (data not shown). However b11 does not require mitochondrial-encoded maturases for its excision[12]. Thus, at least for intron b11, pMSS116 does not intervene in splicing via the synthesis of maturases. These data suggest that pMSS116 is directly involved in the excision of mitochondrial introns.

Recently a second murine initiation factor gene was characterized[13]. Its amino-acid sequence and that of a human protein p68 (ref. 14) is compared with pMSS116 and eIF4A in Fig. 1*a*. Alignment of the four proteins together displays a remarkable conservation of several long amino-acids blocks covering most of the eIF4A sequences (although the middle part is slightly less conserved). Pairwise comparison gives 22.1%, 26.9% and 26.4% identical residues between pMSS116 and p68, eIF4A1 and eIF4A2, respectively. Conservative substitutions have occurred at several other positions. The extreme N-terminal part of pMSS116 has no counterpart in any of the other three proteins. This region is rich in serine, threonine and basic amino acids, and is devoid of acidic residues. These are the characteristics of mitochondrial pre-sequences[15] and support the idea that pMSS116 is imported into the mitochondrion. The eIF4A polypeptide is involved in the melting or unwinding of RNA in the presence of ATP[16,17]. The human p68 polypeptide is supposed to have a DNA helicase activity *in vitro*[14]. This suggests that pMSS116 may also possess a helicase activity.

This suggestion is further strengthened by comparison of the protein sequences with recently reported consensus sequences found in replicative proteins[18]. These proteins include the uvrD and rep helicases as well as recB, UL5 and related proteins which are likely to possess helicase activity (see ref. 18 and references therein). Out of the seven domains described by Hodgman in these helicase and replicative proteins, five are clearly identifiable in pMSS116, p68 and eIF4A (Fig. 1*b*). Blocks I and II probably form part of the ATP binding site, whereas

Table 1 Mitochondrial intron contents of the *cob* and *cox1* genes, and the respiratory competence of strains carrying the *mss116-1* or *mss116-2* alleles

	Mitochondrial intron contents													Respiratory competence	
		cob					*cox1*							allele	allele
Origin of mitochondria	ω	*b*1	*b*2	*b*3	*b*4	*b*5	*a*1	*a*2	*a*3	*a*4	*a*5α	*a*5β	*a*5γ	*mss116-1*	*mss116-2*
(*a*) M12-54	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−
(*b*) GF204-1D	+	−	−	−	+	+	−	+	+	+	+	+	+	−	ND
(*c*) D273-10B/A	+	−	−	−	+	+	+	+	+	+	−	−	+	−	−
(*d*) D273-10B/G1/356-R6	+	−	−	−	+	+	−	−	+	+	−	−	+	+	+
(*e*) GF134-6D	+	−	−	−	+	+	−	−	−	+	−	−	−	ND	+
(*f*) UVA32	−	−	−	−	+	+	−	+	+	−	−	−	+	+	+
(*g*) GF167-7B	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+

Strains indicated in the column 'origin of mitochondria' (GF204-1D excepted) are described in ref. 6. Mitochondria of these seven strains were introduced by cytoduction, via strain K5/2 (ref. 6), either in strain BS7-76-2/2 carrying allele *mss116-1* or in strain D273-M3A/A179/F11 carrying allele *mss116-2*. These three latter strains had their mitochondrial genome deleted (rho°) (ref. 6). Mitochondria intron contents: ω is the 21S ribosomal RNA intron, b1 to b5 are the cytochrome b introns (b1) and a1 to a5γ are the cytochrome oxidase subunit 1 introns (a1); + or − indicates that the intron is present or deleted. Introns b1, a1, a2 and a5γ belong to group II, the others to group I (compare with ref. 20 and references therein). Respiratory competence was tested on glycerol plates: + or −, growth or absence of growth; ND, not determined. Assuming that a block of the splicing of a1$5\alpha$ and/or a1$5\beta$ does not hamper the splicing of any of the five cob introns, we can reach the following conclusions. Mutation *mss116-1* in strain harbouring the mitochondria of D273-10B/A impedes the splicing of *cox1* pre-mRNA (ref. 7), whereas the cytochrome *b* protein is synthesized[11,25]; comparison of lines *a* and *c* implies that *MSS116* is necessary for the splicing of intron b11, b12 and/or b13. By comparing lines *c*, *d* and *f* and knowing that in the D273-10B/A mitochondrial context the splicing of intron a11 is severely deficient (data not shown) we can conclude that *MSS116* is necessary for the splicing of intron a11 and/or a1$5\alpha$ and/or a1$5\beta$. Comparison of lines *c* and *b* leads to the conclusion that *MSS116* is necessary for the splicing of a11 as well as a1$5\alpha$ and/or a1$5\beta$.

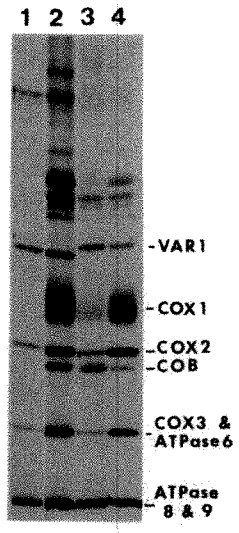


Fig. 2 ^{35}S-labelled mitochondrial translation products of strains, GRF18/B601B (lane 1), GRF18 (lane 2), BS102-1A (lane 3) and BS102-1B (lane 4). Cells were grown in galactose or raffinose complete media. They were labelled with [^{35}S] methionine in the presence of cycloheximide[11]. Radiolabelled mitochondrial translation products were prepared and analysed by SDS-PAGE[24]. Strains GRF18/B601B and BS102-1A bear a disrupted *MSS116* gene (allele *mss116-3*), the others a wild-type *MSS116* gene. Strains GRF18/B601B and GRF18 carry the 13 known mitochondrial introns, strains BS102-1A and BS102-1B have the mitochondria of GF134-6D (compare Table 1). The positions of the mitochondrially synthesized polypeptides are indicated: VAR1, var1 ribosomal protein; cox1, cox2 and cox3, subunits I, II and III of cytochrome *c* oxidase; COB, cytochrome *b*; ATPase 6, ATPase 8 and ATPase 9, subunits 6, 8 and 9 of mitochondrial oligomycin-sensitive ATPase.

blocks Ia, V and VI have only been observed in helicases and related proteins (Fig. 1*b*). These data indicate that the helicase/replicative protein super-family may be extended to include the more divergent eIF4A/p68/pMSS116 subfamily and reinforce the assumption that pMSS116 is an RNA helicase.

Several models may explain how an RNA helicase affects mitochondrial splicing. For example, folding of the nascent transcripts may give rise to several secondary structures in various proportions and only some of these structures will be compatible with a self-splicing activity. In support of this, only a fraction of the RNA present in an *in vitro* self-splicing reaction is active and the transition to the reactive state is the rate-limiting step[19]. An RNA helicase activity, by allowing a cycle of folding and unfolding to take place, will help each molecule reach the active configuration driving the reaction towards splicing of all pre-mRNA molecules. A differential propensity of various introns to non-functional folding will be reflected by different requirements for a helicase.

Another hypothesis is that changes in secondary and/or tertiary structures, which are likely to occur during splicing of mitochondrial introns, may be catalysed by a structure and/or sequence-specific helicase. Thus a folding pattern required early in the splicing process is melted later by a helicase allowing a new structure to form. During nuclear splicing, which is somewhat related to group II intron splicing[20], the U4 and U6 small nuclear RNAs follow a cycle of association and disassociation[21,22], and it has been suggested that a helicase may have a role in melting the two molecules apart[21,23].

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The gene for the U6 small nuclear RNA in fission yeast has an intron

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The small nuclear RNAs (snRNAs) are a class of metabolically stable small RNAs present in the nuclei of eukaryotic cells[1]. In mammalian cells, there are six major molecular species (U1 to U6 snRNA), which are complexed with proteins, forming small nuclear ribonucleoprotein particles, snRNPs. Of these, the U1, U2, U4, U5, U6 snRNPs are thought to participate in pre-mRNA splicing as part of the spliceosome (for review, see ref. 2). Here, we describe the characterization of the gene coding for the *Schizosaccharomyces pombe* U6 snRNA. Unexpectedly, the *Schiz. pombe* U6 RNA gene was found to contain an intron-like sequence of 50 base pairs. Northern blot analysis and RNA sequencing revealed that this intron-like sequence is precisely removed from the transcript. The mature U6 RNA of *Schiz. pombe* has 77% sequence homology with the mammalian U6 RNA. In *Schiz. pombe*, it is possible that U6 RNA is not only involved in pre-mRNA splicing, but is also a splicing substrate. This is the first report of an intron in a snRNA gene.

U6 RNA is one of the six abundant, capped snRNAs present in eukaryotic cells[3-9]. U6 and U4 RNAs are base-paired and are present in the same snRNP[10,11]. The U1, U2, U5, U4/U6 snRNPs assemble onto the pre-mRNA substrate to generate the active spliceosome[2]. U4 RNA is thought to be released from the assembled spliceosome during the splicing reaction[12]. The U1 snRNP interacts with the 5′ splice site, the U2 snRNP with the branch point, and the U5 snRNP may interact with the 3′ splice site. The exact function(s) of the U4/U6 snRNP in pre-mRNA splicing, however, is not known.

To elucidate the function(s) of U6 RNA, we isolated the gene coding for U6 RNA of the fission yeast *Schiz. pombe*, which is as convenient for genetic analysis and gene manipulation as the budding yeast *Saccharomyces cerevisiae*. In addition, it has been proposed that the splicing machinery of *Schiz. pombe* is closer than that of *Sacc. cerevisiae* to the machinery of higher eukaryotes[13].

The nucleotide sequence of U6 RNA is highly conserved among species[14]. We synthesized the 30-mer oligonucleotide complementary to the most conserved region of U6 RNA (nucleotides 40–69 of rat U6 RNA[5]) and used this as a probe, initially for Southern and northern blot analysis of the total

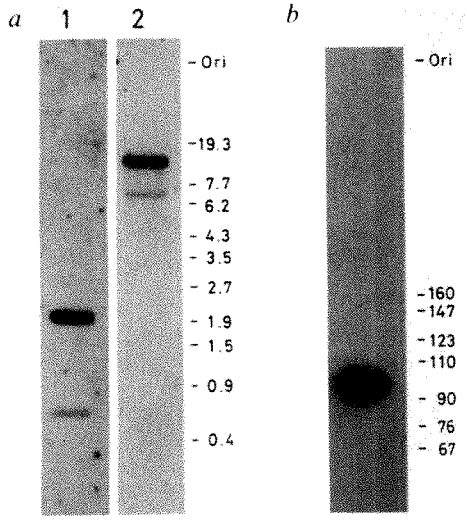
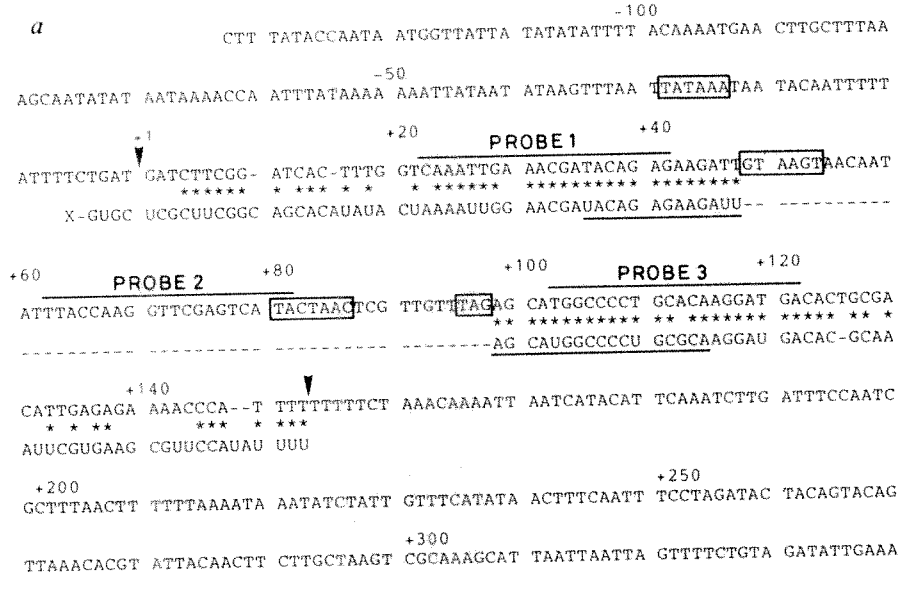


Fig. 1 *a*, Southern blot analysis of total DNA. Total *Schiz. pombe* DNA (10 μg) was digested with *Hin*dIII (lane 1) or *Pst*I (lane 2), electrophoresed on a 1% agarose gel, transferred to a Nylon membrane (Biodyne) and hybridized with the kinase-labelled oligonucleotide. Positions of size markers (λ *Sty*I digest) are on the right in kb. *b*, Northern blot analysis. *Schiz. pombe* total RNA (30 μg) was electrophoresed on a 5% polyacrylamide/7M urea gel, transferred to a Nylon membrane (Biodyne) by capillary action, and probed. Markers were kinase-labelled *Hpa*II fragments of pBR322 DNA.

Methods. Hybridization was at 42 °C in a solution containing 6× SSC, 50 mM sodium phosphate, 5× Denhardt's solution, 0.1% SDS, 2 mM EDTA and 20 μg ml^{-1} tRNA. The filter was washed in 3 M tetramethylammonium chloride, 2 mM EDTA, 50 mM Tris-HCl (*p*H 8.0) at room temperature for 30 min and then at 50 °C for 20 min. Autoradiography was carried out at −70 °C with an intensifying screen.



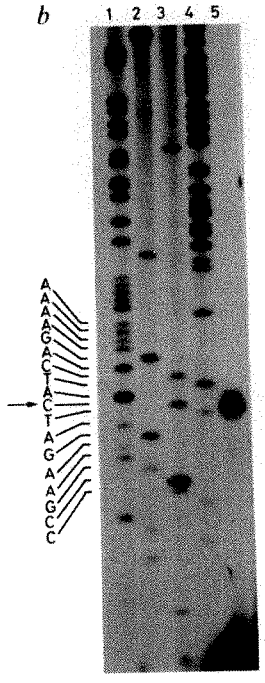


Fig. 2 *a*, Nucleotide sequence of the gene encoding the U6 RNA from *Schiz. pombe*. The upper line shows the sequence of the non-coding DNA strand. The lower line shows the sequence of the rat U6 RNA. Asterisks indicate identical nucleotides and dashes denote gaps introduced to optimize sequence homology. The 5′ and 3′ ends of the coding region were determined as described in the text and indicated by the vertical arrow heads. A presumptive 'TATA box' and the sequences similar to the consensus sequence of donor, acceptor and branch site are boxed. Numbers indicate distance in nucleotides from the predicted transcription initiation site. Overlines represent the sequences complementary to the probes used for northern blot analysis. Underlines indicate U6 RNA sequence (+40 to +69) complementary to the hybridization probe used for cloning. *b*, Mapping of the 5′ end of the coding region. The oligonucleotide complementary to the region +21 to +40 (probe 1) was used for primer extension reactions with total *Schiz. pombe* RNA (lane 5). The products of sequencing reactions using the same primer were run on lanes 1–4. The DNA sequence is written to the left of the panel. Primer extension reactions were performed as described[22].

Schiz. pombe DNA and RNA. Autoradiography of a Southern blot showed a single major hybridizing band of 2.0 kilobases (kb) and 9.3 kb and a minor band of 0.8 kb and 6.8 kb in the *Hin*dIII and *Pst*I digests, respectively (Fig. 1*a*). Similarly, a single major band of 100 nucleotides, close in size to that of mammalian U6 RNA, was detected by northern blot analysis (Fig. 1*b*). These results indicate that a gene which has strong homology with the probe is present at a single copy in the *Schiz. pombe* genome. We therefore constructed a genomic library derived from 1.5–2.5 kb *Hin*dIII fragments of *Schiz. pombe* DNA, and screened the library with the oligonucleotide probe. Positive clones were analysed by restriction mapping and nucleotide sequencing. All the positive clones analysed contained the same 2.0 kb fragment. Figure 2*a* shows the nucleotide sequence of the region that hybridized with the probe. A single U6 RNA gene was found in the 2.0 kb fragment. The 5′ end of the coding region was determined by primer extension (Fig. 2*b*) and RNA sequencing using the dideoxy method (data not shown). The 3′ end was tentatively assigned to be at +149 by a comparison with rat U6 RNA sequence. Although all U6 RNA genes cloned to date have a TATA box in the 5′-flanking region, several lines of evidence indicate that U6 RNA is transcribed by RNA polymerase III[3,6,15,16]. The putative 3′ end of the *Schiz. pombe* U6 RNA gene falls within a (T)-residue tract characteristic of transcription termination sites of RNA polymerase III.

Comparison of the nucleotide sequence of the coding region with that of rat U6 RNA revealed an unexpected structure of the *Schiz. pombe* U6 gene. Sequence identity with rat U6 RNA is abruptly interrupted at +47, and is resumed at +97. Interestingly, the 5′ and 3′ ends of the interruption are GTAAGT and TAG, respectively. These are identical to the consensus sequences at the ends of introns of all eukaryotic genes[17]. In addition, the sequence 5′-TACTAAC 3′, which is located 12 base pairs (bp) upstream of the 3′ boundary, matches the potential branch site sequence 5′-CTRAY-3′ present 6–18 residues upstream of the 3′ splice site of *Schiz. pombe* genes[18]. More remarkably, this sequence is identical to the TACTAAC box, the strictly conserved branch-site sequence found in *Sacc. cerevisiae*[19,20]. The distance between GT and AG is similar to the length of many *Schiz. pombe* introns. All these structural features suggest that the region +47 to +96 is an intron.

To investigate whether the intron-like sequence is actually an intron, we performed several experiments. First, northern blot analysis was carried out using the oligonucleotides complementary to three parts of the gene as probes (see Fig. 2*a*). As shown in Fig. 3*a*, probes 1 and 3 hybridized to RNA of the same size (about 100 nucleotides). In contrast, no hybridizing band was detected with probe 2, which is complementary to the intron-like region. These results suggest that the middle part of the transcript has been removed. Second, the partial nucleotide sequence of the *Schiz. pombe* U6 RNA was determined by the dideoxy method to localize the precise splice site (Fig. 3*b*). The nucleotide sequence of the mature U6 RNA indicates that the region +47 to +96 is indeed spliced out from the RNA. The U6 RNA sequence determined (68 nucleotides from the 5′ end) was completely co-linear with the gene sequence, except for the region +47 to +96. Furthermore, the size of the mature product (99 nucleotides) predicted from the gene sequence agrees well with the result of northern blot analysis. Third, we used Southern blot analysis with 12 restriction enzymes and probe 1 to exclude the possibility that there is an intron-less U6 gene. A single hybridizing band was detected in all cases, confirming that the *Schiz. pombe* U6 RNA gene is unique in the genome (data not shown). The minor bands (0.8 kb and 6.8 kb in *Hin*dIII and *Pst*I digests, respectively) were not detected with probe 1 even after a long exposure. Therefore, the minor band observed with the 30-mer probe does not seem to represent a U6 gene. Fourth, accumulation of a putative U6 RNA precursor of about 150 nucleotides was observed in *Schiz. pombe* which had been incubated at 43 °C for 30 min before RNA extraction (unpublished results). On the basis of these results we conclude that the *Schiz. pombe* U6 RNA gene has an intron.

The nucleotide sequence of U6 RNAs are highly conserved among species (Fig. 4). The mature *Schiz. pombe* U6 RNA has a 77% sequence identity with mammalian U6 RNA. The intron is present within the most conserved region of U6 RNA, the region thought to be important for U4:U6 interaction[10].

To our knowledge, this is the first example of an snRNA gene containing an intron. Although various snRNA genes have been cloned, none were found to have an intron. As for U6 RNA genes, introns have never been detected in genes from mammals[3,21], *Drosophila melanogaster*[9], *Xenopus tropicalis*[6] and the budding yeast *Sacc. cerevisiae*[7]. If *Schiz. pombe* U6 RNA is indeed a RNA polymerase III transcript, as it is in other organisms, the *Schiz. pombe* U6 RNA gene is the first gene to be transcribed by RNA polymerase III to have a typical mRNA intron. In the *Schiz. pombe* U6 RNA gene, the region corresponding to a 'box A' intragenic control sequence (5′-AGATTAG-CATGG-3′) of RNA polymerase III is disrupted by the intron

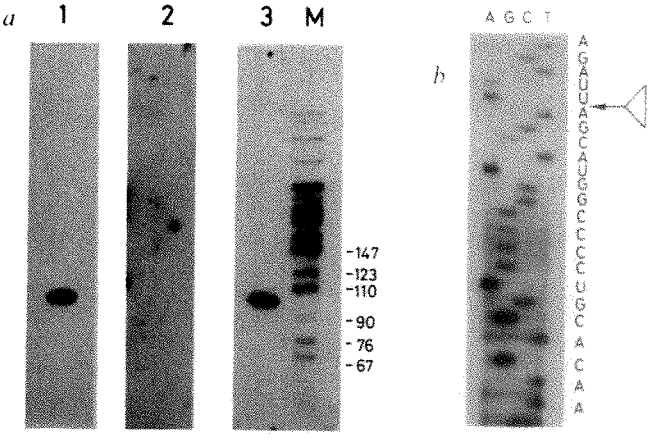


Fig. 3 *a*, Northern blot analysis. *Schiz. pombe* total RNA (30 μg) was electrophoresed on a 5% polyacrylamide/7 M urea gel, transferred to a Nylon membrane (Biodyne), and probed separately with the probe 1 (lane 1), probe 2 (lane 2) and probe 3 (lane 3). Hybridization conditions were the same as described in the Fig. 1 legend. Markers were kinase-labelled *Hpa*II fragments of pBR322 DNA (lane M). *b*, Sequence near the splice junction of the *Schiz. pombe* U6 RNA. The oligonucleotide complementary to the region +121 to +140 was kinase-labelled, and annealed to 30 μg of total *Schiz. pombe* RNA. The sample was split into four aliquots and used to direct cDNA synthesis[22] in the presence of ddATP (lane A), ddGTP (lane G), ddCTP (lane C), or ddTTP (lane T). Samples were run on a 8% polyacrylamide/8.3 M urea gel. The RNA sequence predicted by the cDNA sequence is written on the right side of the panel. The arrow shows the position where the intron sequence was located.

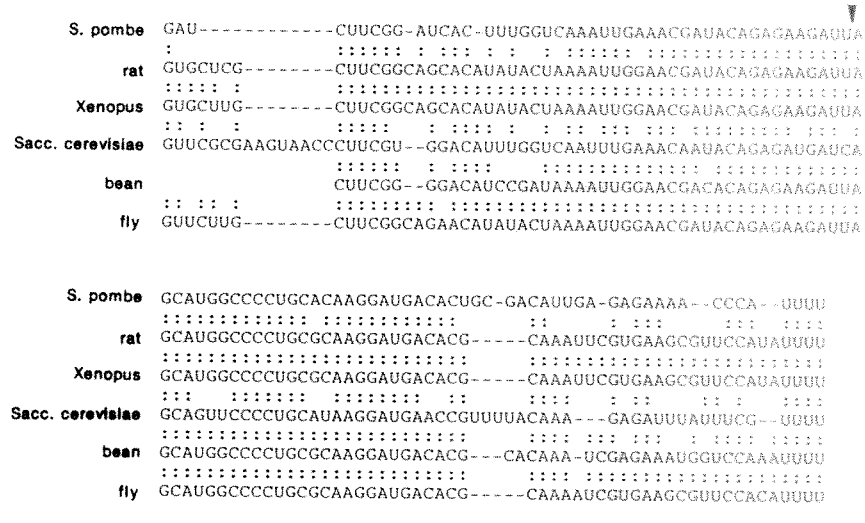


Fig. 4 Comparison of primary structures of U6 RNAs. The nucleotide sequence of the mature *Schiz. pombe* U6 RNA is aligned with that of U6 RNA from the rat[5], *Drosophila melanogaster*[9], *Xenopus tropicalis*[6], *Sacc. cerevisiae*[7], and broad bean (*Vicia faba* L.)[8]. Colons indicate the nucleotides identical to those of rat U6 RNA. Dashes represent gaps introduced to improve the alignment. The vertical arrowhead indicates the position of the intron present in the *Schiz. pombe* U6 RNA gene.

sequence. This sequence, however, is dispensable for efficient and accurate transcription of the vertebrate U6 RNA gene[15]. In addition, there is no consensus polymerase III intragenic sequence in the budding yeast U6 RNA gene, which is transcribed by yeast polymerase III[7].

It is surprising that the U6 RNA gene of fission yeast, with such a small genome size, has an intron. We do not know whether the intron of the U6 RNA gene has been removed in other organisms, or whether an intron sequence has jumped into the U6 RNA gene of *Schiz. pombe* during evolution. Oligonucleotide probe 2, which is complementary to the intron region gave a single major band corresponding to the cloned gene in Southern blots of the total *Schiz. pombe* DNA (data not shown). This would indicate that the U6 intron sequence is unique in the *Schiz. pombe* genome. It is, therefore, unlikely that the U6 intron is a kind of transposon.

It is possible that the *Schiz. pombe* U6 RNA is involved in RNA splicing as an RNA component of the spliceosome although U6 RNA itself is a substrate for RNA splicing. Probe 2 did not detect a band corresponding to the U6 RNA precursor on a northern blot, suggesting that the precursor is spliced very rapidly. If U6 RNA is a component of the splicing machinery, it must be very efficiently spliced, and thus must have the most efficient splicing signals. It is noteworthy that the intron of the *Schiz. pombe* U6 RNA gene has TACTAAC sequence, identical to the branch-site sequence of *Sacc. cerevisiae*.

The budding yeast gene for U6 RNA (SNR6) is reported to be essential for cell viability[7]. Gene disruption experiments are in progress to investigate whether the U6 RNA gene is essential for *Schiz. pombe*.

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Structural differences between a *ras* oncogene protein and the normal protein

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One of the most commonly found transforming *ras* oncogenes in human tumours has a valine codon replacing the glycine codon at position 12 of the normal c-Ha-*ras* gene[1–4]. To understand the structural reasons behind cell transformation arising from this single amino acid substitution, we have determined the crystal structure of the GDP-bound form of the mutant protein, p21(Val-12), encoded by this oncogene. We report here the overall structure of p21(Val-12) at 2.2 Å resolution and compare it with the structure of the normal c-Ha-*ras* protein[5]. One of the major differences is that the loop of the transforming *ras* protein that binds the β-phosphate of the guanine nucleotide is enlarged. Such a change in the 'catalytic site' conformation could explain the reduced GTPase activity of the mutant[6–8], which keeps the protein in the GTP bound 'signal on' state for a prolonged period of time, ultimately causing cell transformation.

The cloning and expression[9] of the synthetic gene coding for human c-Ha-*ras* p21 proteins and the crystallization conditions[10] have been described. Briefly, the synthetic gene (using *Escherichia coli* preferred codons) for amino-acid residues 1–171 was cloned and expressed in *E. coli*. We have deleted the codons corresponding to the carboxy-terminal 18 residues because there

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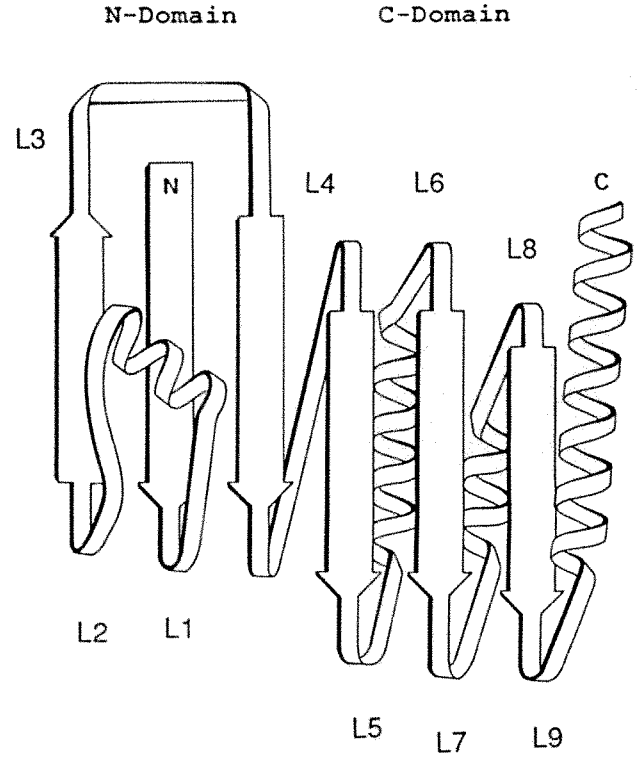


Fig. 1 Topological structure of human c-Ha-*ras* p21 protein. Each β-strand, α-helix and loop is numbered starting from the amino-terminus. The N-domain, which covers three β-strands and one α-helix, is barely hydrogen-bonded to the C-domain. The former has much higher thermal motion than the latter, suggesting that the flexibility of the N-domain is greater. The catalytic site for GTP hydrolysis is localized in the N-domain (phosphate binding domain) and the recognition for guanine base is in the C-domain (guanine recognition domain).

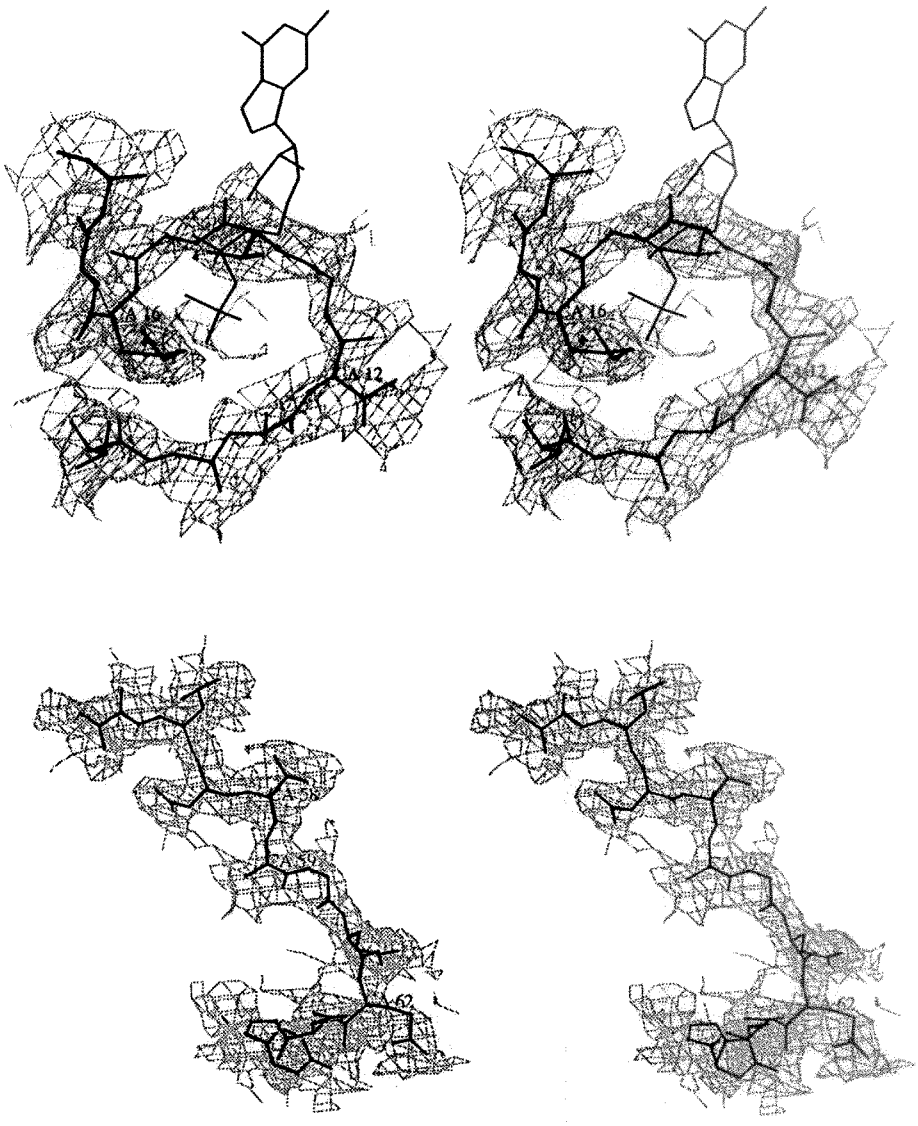


Fig. 2 Electron density maps for p21 (Val-12) around loop L1 and part of loop L4 with model fitting. These portions of electron density have been calculated with phases obtained from all atoms in the molecule, omitting those belonging to residues 9–18 and 55–64. The conformation of both regions in this mutant is different from that of normal p21.

is reasonable evidence that this region is flexible[11,12], and this flexibility could be detrimental to obtaining good quality crystals. Only the soluble fraction of the protein was purified for crystallization. The GTPase activity of p21(Val-12, 1–171) is less than 10% of that of the normal protein. High-quality single crystals were obtained from solutions containing 10 mg ml^{-1} protein, 0.1 M $CaCl_2$, 0.075 M HEPES buffer at *p*H 7.5, 0.5 mM EDTA, 0.5 mM dithiothreitol and 0.005% *n*-octyl glucoside, equilibrated to 30% PEG 400. The crystals are isomorphous with those of the normal protein, and have space group $P6_522$ with cell parameters $a = b = 83.2$ Å and $c = 105.1$ Å.

The diffraction data for p21(Val-12, 1–171) were collected on an Enraf Nonius rotation camera installed on the 8-pole wiggler line at the Stanford Synchrotron Radiation Laboratory, Palo Alto, California. The X-ray wavelength used for data collection was 1.08 Å, the crystal-to-film distance was 85 mm, and 2° rotation was used for each exposure. One set of data was collected from one crystal at 4 °C on a total of 32 films (16 film packs). The films were digitized on a drum scanner (P-1000, Optronics), and processed with a program originally written by Rossmann[13]. The reflection data from different films were then merged using the PROTEIN program package[14]. A total of 48,073 observations were used in the merging process, giving 10,447 unique reflections to 2.2 Å resolution, with an overall R(merge) on intensity of 8%.

The crystallographic refinement with the TNT program package[15] used the model for the normal protein[5] as a starting point.

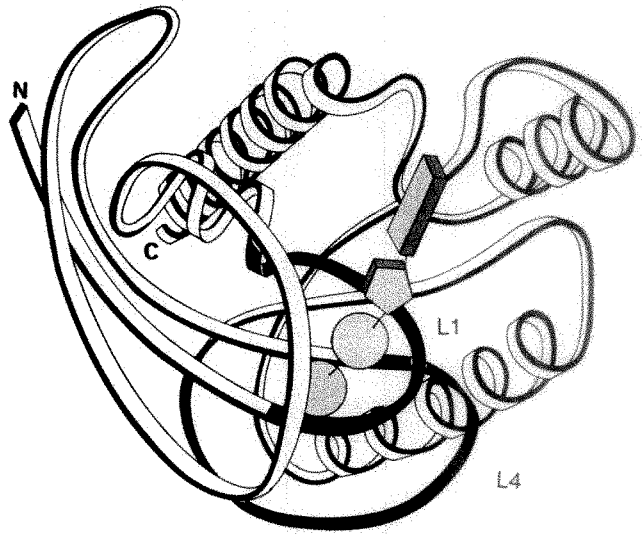


Fig. 3 An artist's drawing, looking down the guanine nucleotide-binding pocket, of the transforming human c-Ha-*ras* oncoprotein p21(Val-12) crystal structure. Cylinders, ribbons and thick black curves represent α-helices, β-strands and loops, respectively. The bound GDP molecule is identified by stippled shapes. The four loops L1, L2, L3 and L4 (Fig. 1) with conformation different from those in the normal protein are labelled.

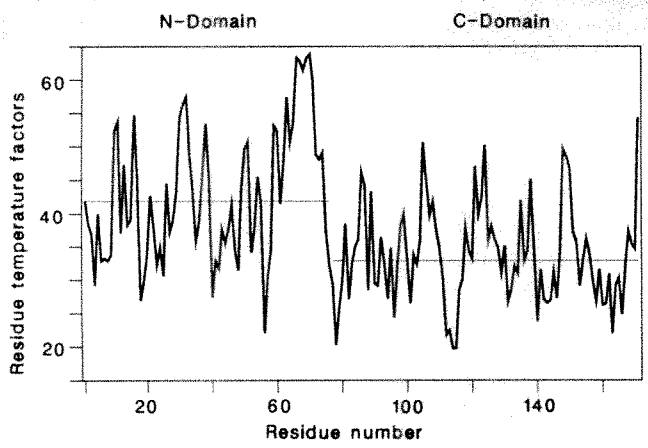


Fig. 4 Average main-chain temperature factors for p21(Val-12). The thin horizontal lines represent the average main-chain temperature factors for the N-terminal (1-75) and C-terminal (76-171) residues. Notice that the temperature factors for residues 60-75 of loop L4 are the highest in the structure.

During the course of refinement, omit maps were calculated by removing residues 9-18 and 55-64, corresponding to loop L1 and part of L4 (Fig. 1), and the model was refitted to the omit maps. Examples of such omit maps are shown in Fig. 2. The current R factor for 9,053 reflections between 10 and 2.2 Å resolution (80% of total possible) is 23.6%. A detailed description of the refinement and the refined structure will be reported elsewhere.

The overall structure (Fig. 3) of transforming p21(Val-12) is similar to that of the normal protein[5]. It contains six β-strands, four α-helices, and nine connecting loops (Fig. 1). Both structures seem to consist of two recognizable domains: the amino-terminal domain, containing the first 75 residues (including the first three β-strands and one α-helix), is the phosphate binding domain, and the carboxy-terminal domain, containing the remaining residues (including the last three β-strands and three α-helices), is the guanine recognition domain. There is only a short stretch of hydrogen bonding between β3 in the N-terminal domain and β4 in the C-terminal domain (Fig. 1). This separation of domains is also manifested by the distribution of the residue temperature factors in each domain (Fig. 4). The residues in the N-terminal domain have higher temperature factors (an average of 42 Å^2), and thus are more mobile than those in the C-terminal domain (an average of 33 Å^2). This high mobility of the N-terminal domain may have a functional significance in that it is in this domain where the catalytic site of GTP hydrolysis, the putative effector region[16,17], and the GTPase-activating protein-binding region (residues 30-40)[18,19] are located.

The differences between the two structures are mainly localized in the loops L1, L2, L3 and L4 in the N-terminal half of the molecule (Figs 1 and 3). The root-mean-square difference in Cα between p21(Val-12) and the normal protein is 1.26 Å for residues in the N-terminal domain (residues 1-75) and 0.56 Å for residues in the C-terminal domain (residues 76-171). At the current stage of the refinement, the two regions with the largest differences are located in loops L1 and L4. However, the electron density for residues in loop L4 is not as well-defined (as evidenced by their high-temperature factors, Fig. 4). Further refinement is required to assess the significance of the differences in this region.

The clearest and largest differences in structure were found in loop L1, corresponding to residues 9-18, which wraps around the β-phosphate of the bound GDP molecule (Fig. 5). The current refinement results from the normal protein, as well as from transforming p21(Val-12), show that there are several unusual features in this loop (Fig. 5). First, there seem to be no side chains, except that of Lys-16, involved in binding to the phosphate. Second, the backbone amide groups are pointing towards the β-phosphate, making hydrogen bonds to the phosphate oxygens and thus fixing the orientation of the β-phosphate, which is presumably important for GTPase activity. Third, there is a metal ion (probably Mg^{2+} or Ca^{2+}) coordinated to the oxygen atoms of the β-phosphate.

The simplest description of the structural differences in loop L1 is that the size of this loop in p21(Val-12) is much larger than in the normal protein (Fig. 5), resulting in the loss of two hydrogen bonds (from the backbone NH groups of residues 12 and 13) to the β-phosphate. We have suggested[5] that this loop would have straddled the phosphodiester bond between the β- and γ-phosphates of GTP, and therefore is the prime candidate to be the catalytic site for GTP hydrolysis in the normal p21 protein. The loss of two hydrogen bonds may alter the orientation of the β-phosphate, either when presented to the attacking group, or as a leaving group after the γ-phosphate is attacked, thereby changing the GTP hydrolysis rate. The position of the bound metal ion is such that it is a reasonable candidate for providing the attacking group (probably one of its bound water molecules) to the β-phosphate, perhaps for the on-line displacement of the γ-phosphate. Alternatively, the cation may be participating in fixing the β-phosphate in collaboration with backbone NH groups, with an as-yet-unidentified water molecule attacking the β-phosphate. Another mechanism, similar to that proposed for elongation factor Tu (ref. 20), is

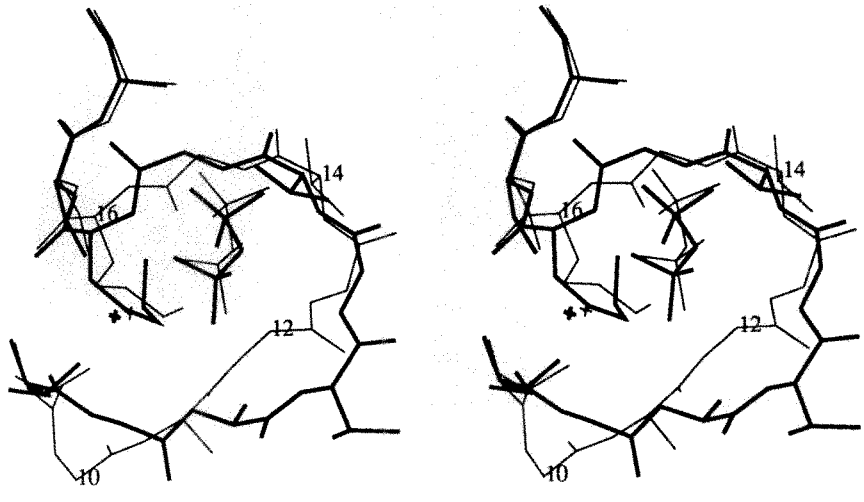


Fig. 5 A stereo view showing the conformation of Loop L1. The thick line is the conformation of loop L1 in p21(Val-12) and the thin line represents that of normal p21 protein. The small crosses represent the positions of the metal ions, and two phosphate groups are shown. Notice that the loop conformation around the β-phosphate in normal p21 protein is much tighter than that of p21 (Val-12).

that the γ-phosphate (which is not present in our structure) is attacked, possibly by a water molecule. This could explain the autophosphorylation activity of the viral *ras* proteins[21], because in that case the hydroxyl group of Thr-59 of viral p21, located near the presumed γ-phosphate site, could be the attacking group.

In summary, the crystal structure of transforming c-Ha-*ras* p21(Val-12, 1–171) with a bound GDP molecule provides a plausible explanation for the loss of GTPase activity of the protein, thus keeping the protein in the GTP-bound state, which is thought to signal continued cell proliferation without regulation. A complete understanding will have to await the structure determination of GTP bound c-Ha-*ras* p21 and of different activated mutants. Efforts to obtain p21 crystals with bound, non-hydrolysable GTP analogue and to determine the structure of the activated mutant p21(Leu-61, 1–171) are in progress. The coordinates will be deposited in the Brookhaven Protein Data Bank.

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Structure of the membrane-pore-forming fragment of colicin A

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Colicins are antibiotic proteins produced by and active against sensitive *Escherichia coli* and closely related bacteria. They can adsorb to specific receptors located at the external surface of the outer membrane of sensitive cells, and are then translocated to their specific targets within these cells. The largest group of colicins comprises those which can form voltage-dependent channels in membranes, thereby destroying the cell's energy potential[1]. Colicin molecules are organized in structural domains, each domain carrying one function associated with the toxin's lethal activity. The pore-forming activity seems to be located at the carboxyl terminus. A thermolytic fragment comprising amino acids 389–592 from colicin A has pore-forming properties very similar to those of the entire molecule. This fragment is soluble in aqueous medium and spontaneously inserts into lipid bilayers[2]. We have determined the structure of the pore-forming fragment of colicin A by X-ray crystallography and refinement at 2.5 Å resolution. The protein consists of ten α-helices organized in a three-layer structure. Two of the helices are completely buried within the structure and form a hydrophobic hairpin loop similar to that proposed for signal sequences which function in translocation. We present a model for insertion of the protein into lipid bilayers the features of which may be applicable in other biological systems involving protein insertion or translocation across membranes.

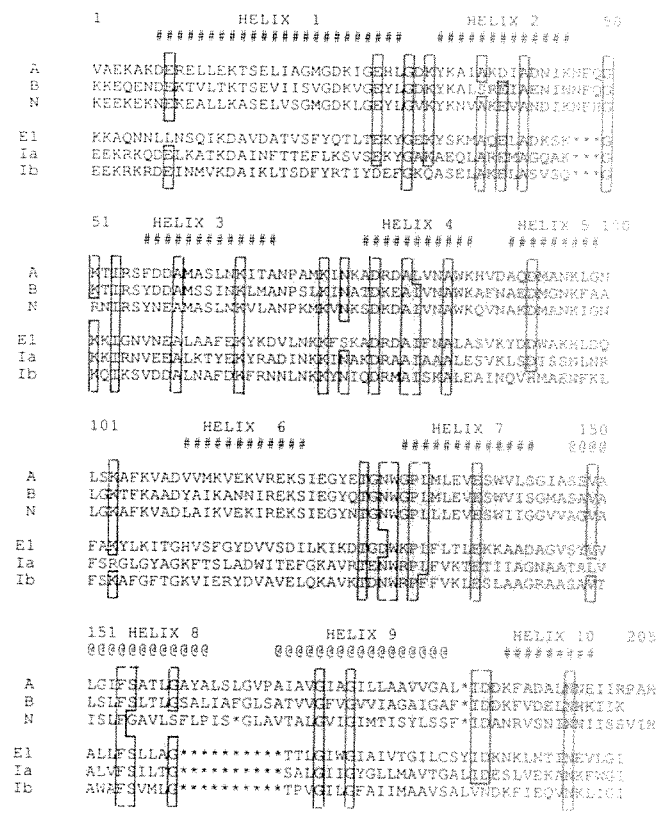


Fig. 1 Aligned sequences of the pore-forming domain of colicins. These sequences can be subdivided into two groups on the basis of sequence identity. Highly conserved residues are boxed. The position of alpha-helices in the sequence are shown. The # symbol refers to surface helices and the @ symbol to buried helices.

We have previously reported the crystallization of the thermolytic fragment of colicin A (ref. 3). The crystals belong to space group $P4_32_12$ with cell parameters $a = b = 73.0$ Å, $c = 171.3$ Å. There are two monomers per asymmetric unit. Diffraction data were measured on a multiwire area detector (Nicolet/Xentronics) mounted 20 cm from the crystal on a rotating anode X-ray generator (Elliot GX-18). The data sets were processed using the Genex software package[4]. Over thirty attempts to obtain an isomorphous derivative of the protein crystals using heavy-atom reagents were unsuccessful. Because the wild-type thermolytic fragment of colicin A contains no cysteines, and because mercurial reagents bound to cysteine residues often yield good isomorphous derivatives, we decided to use site-directed mutagenesis to construct cysteine mutants of the proteins (manuscript in preparation). The best heavy-atom derivative was made by soaking in mercury chloride the crystals of a mutant that had a serine replaced by a cysteine (Ser 16 → Cys, residue numbering as in Fig. 1) and a repeat decapeptide inserted just before it. The phase set constructed from this derivative was subsequently improved by application of solvent flattening techniques[5,6]. An atomic interpretation of the electron density map calculated at 3.0 Å resolution was constructed and refined using a combination of molecular dynamics[7,8] and the distance-

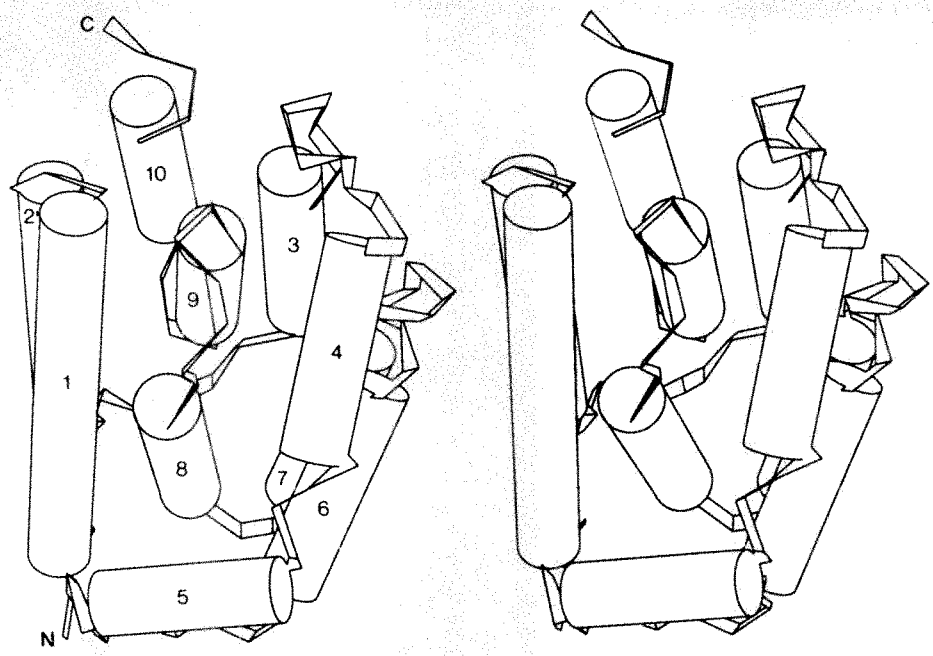


Fig. 2 Stereo representation of the chain fold of the pore-forming fragment of colicin A. Alpha-helices are represented by cylinders and are labelled 1 to 10. This figure was produced by a computer program written by Lesk and Hardman[27].

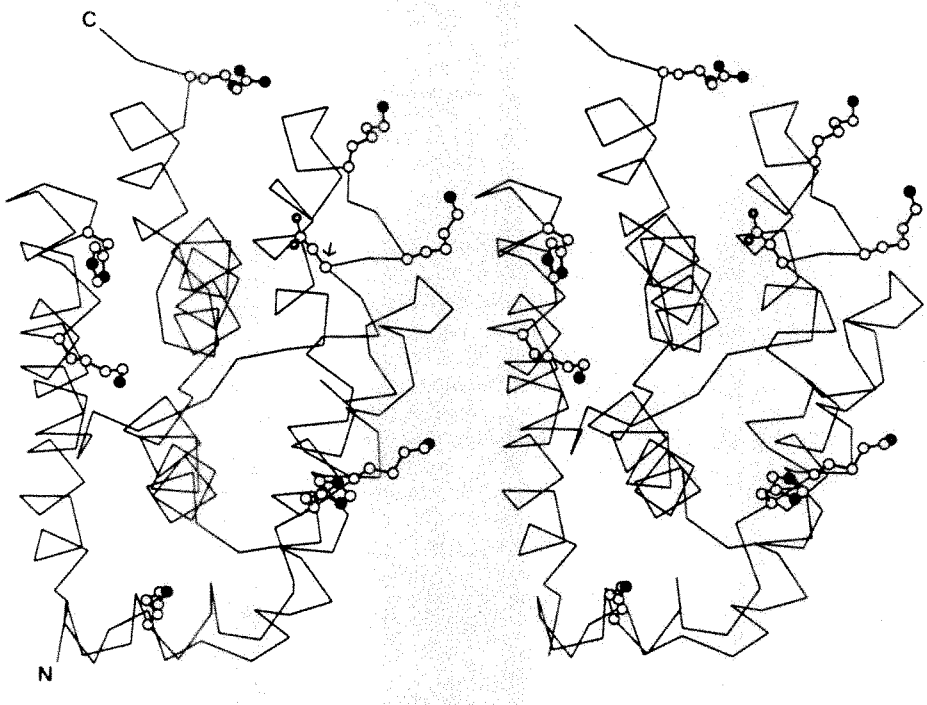


Fig. 3 Stereodiagram of an α-carbon representation of the chain fold showing the array of charged residues on the protein interface thought to interact with membranes. All residues are positively charged with the exception of Asp 78, which is indicated by an arrow. This figure was produced by a computer program written by Lesk and Hardman[27].

restrained least-squares procedure of Konnert and Hendrickson[9]. The current crystallographic *R*-factor of the refined model is 24.4% (10,024 reflections $> 3\sigma$, 6.0–2.5 Å, overall temperature factor). The r.m.s. deviation from ideal bond lengths is 0.024 Å. Details of the crystallographic work will be published elsewhere. For ease of discussion the terms 'thermolytic' or 'pore-forming' will be used interchangeably.

The structure of the pore-forming fragment of colicin A is presented in Fig. 2. The molecule can be described as a bundle of ten α-helices which are arranged in three layers. The layer containing the amino terminus of the peptide fragment consists of a pair of antiparallel amphipathic helices (helices 1 and 2). This N-terminal layer is connected by a long loop to another layer which consists of two pairs of antiparallel amphipathic helices (helices 3 and 4 and helices 6 and 7) with a helix (number 5) connecting the two helix pairs. The middle layer consists of three helices (helices 8, 9 and 10), the first two of which are completely buried and consist of hydrophobic residues only. The peptide fragment crystallizes as a dimer in the asymmetric unit of the unit cell. It should be noted that the protein normally exists as a monomer in solution and the monomeric state is considered to be the physiologically relevant state of the water-soluble form of the protein[10]. The dimer interface consists of contacts from the C-terminal end of helices 1, 3, 8 and 10, and from the N-terminal end of helices 2, 4 and 9, as well as contacts from residues in loops connecting these helices. Figure 2 has

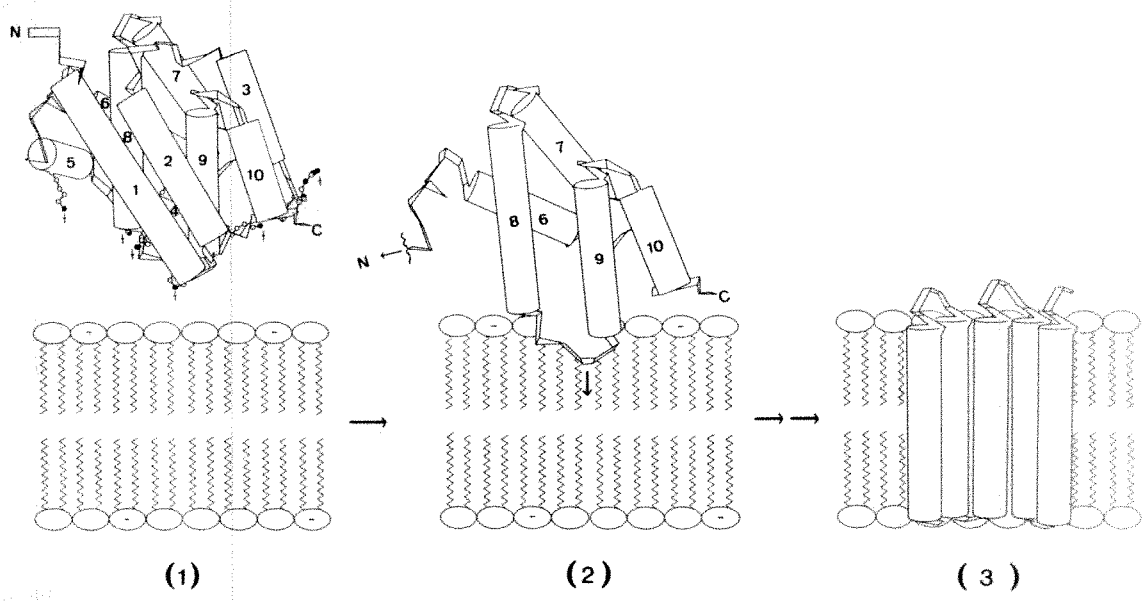


Fig. 4 A model for protein insertion into membranes suggested by the structure of the pore-forming fragment of colicin A. (1) Initial interaction with the membrane occurs as a result of the long-range interaction of an electrostatic potential field and shorter-range interactions such as salt bridges. The first step orientates the hydrophobic helical hairpin (helices 8 and 9) so that its axis is perpendicular to the membrane surface. (2) The hairpin spontaneously penetrates into the membrane, and in doing so initiates a conformational change in the protein. (3) It is envisaged that further steps involve insertion of other helical hairpins which may include oligomerization of the protein molecule. These final steps would lead to the formation of a voltage-gated channel.

been drawn so that this interface appears in the foreground plane of the diagram. The pore-forming fragment of colicin A is the second example of a three-layer α-helical fold first defined by citrate synthase[11]. There is no resemblance between the two proteins as far as structural details or function are concerned however.

The paradox that a colicin can exist as both a water-soluble protein and as a membrane protein is resolved by the structure of the pore-forming fragment of colicin A. Colicin A and its thermolytic fragment are soluble in water and monomeric over a wide range of *p*H and concentration conditions[10]. But the sequences of colicin A and related colicins all contain a hydrophobic stretch of residues between 35 and 49 amino acids long (Fig. 1). This stretch of sequence would be classified as a membrane-spanning sequence on the basis of hydropathy profiles[12]. In the three-dimensional structure, this membrane-spanning sequence adopts a helical hairpin motif (helices 8 and 9) which is completely buried within the protein. Thus residues that interact with lipid in the membrane-bound form of the protein are buried within the protein conformation of the water-soluble form.

The mechanism of insertion of a protein into a lipid bilayer can be divided into two steps: binding to the membrane surface and insertion of the protein into the hydrophobic core of the bilayer. The first step is probably electrostatic in nature, whereas the second may be driven by the partitioning of hydrophobic residues into the aliphatic environment of the bilayer. The electrostatic nature of the binding is supported by the following observations. Colicin A and its thermolytic fragment do not bind to neutral bilayers but display a great affinity for negatively charged lipids[13]. Binding can occur without insertion below the lipid phase transition. Penetration of the bilayer is optimized at acidic *p*H (the structure of the thermolytic fragment was determined at *p*H 4.4). The pore properties of colicin A suggest it is asymmetrically orientated with respect to the membrane. Analysis of the distribution of surface side chains of the pore-forming peptide structure shows that they are randomly distributed with respect to various potential interaction parameters such as charge, hydrophobicity and aromaticity. The exception to this finding occurs at the surface defined by the loop that connects helices 8 and 9 in the structure (Fig. 2). The loop is surrounded by a ring of eight positively charged side chains (residues 25, 29, 73, 76, 87, 88, 97 and 201; see Fig. 3). The only negatively charged residue on this surface is Asp 78 which is located inside the ring of positively charged residues (Fig. 3). It is striking that the interface defined by this loop and surrounding side chains is the same interface used in formation of the non-crystallographic dimer observed in the unit cell of the crystal. Thus this interface appears to have the potential to bind to surfaces found on proteins or lipid bilayers. The mean positive charge of the interface explains the affinity of colicin for negatively charged lipids.

The proposed orientation of binding of the pore-forming domain positions the hydrophobic helical hairpin directly above the membrane surface with helices 8 and 9 sitting perpendicular to the plane of the bilayer. Figure 2 has been drawn such that the proposed interaction surface of the peptide with the bilayer is placed in the foreground plane of the diagram. The positioning of the helical hairpin and the hydrophobic nature of the exposed loop connecting the two helices together with the loss of solvation at the peptide-membrane interface on binding promotes the insertion of the hairpin into the hydrophobic core of the bilayer. This implies that the structure must undergo significant conformational changes because the hairpin constitutes the hydrophobic core of the water soluble form of the protein. There is some evidence that supports a partial unfolding of colicin upon insertion into bilayers. Lowering the dielectric constant of the aqueous solvent with ethanol promotes the partial collapse of the far ultraviolet circular dichroism spectrum of the pore-forming peptide, whereas further addition of ethanol reverses the trend[12]. A similar effect is observed when the peptide is added to various concentrations of dimyristoylphosphatidyl glycerol vesicles at acidic *p*H (F. P., unpublished results). There is evidence of a drastic change in the mobility of some side chains on binding to lipid vesicles, as observed by circular dichroism spectroscopy in the near ultraviolet region (F.P., unpublished results). Proteolysis experiments indicate that the C-terminal helix does not get inserted, but is retained on the insertion side of the lipid bilayer[14].

At this stage there are not enough data to predict the protein

topology when inserted into the membrane. But it is noteworthy that the structure of the pore-forming fragment shows that helices are organized in a pairwise fashion. It is tempting to speculate that the insertion occurs by a stepwise insertion of helical hairpins without large changes in secondary structure conformation. The α-helical content of the water-soluble and lipid-bound form of the peptide fragment is identical, as judged by circular dichroism[12]. Mutation studies indicate that as few as 130 amino acid residues from the C-terminal end of the peptide are sufficient for pore formation[15]. A similar study of the homologous colicin E1 indicates that only the last 88 amino acids are required for pore formation[16]. These studies suggest that the insertion of the first helical hairpin (helices 8 and 9) into the membrane provides the energy for insertion of other helical hairpins. After insertion of the first helical hairpin, a large degree of hydrophobic association energy between the other helical hairpins would be lost, making the insertion of further hairpins more energetically favourable. The insertion of the first hairpin would bring the other hairpins closer to the membrane surface and hence make them more responsive to any electrochemical potential effects. Model-building suggests that the pore is probably formed by an oligomer of colicin molecules. An oligomerization process could have an important role in the insertion of other helical hairpins by shielding their polar faces from contact with the hydrophobic core of the membrane and forming the water-filled pore of the voltage-gated channel in doing so. Key features of the model for membrane insertion are presented in Fig. 4.

The three-dimensional structure of the pore-forming fragment of colicin A reveals a number of features relevant to the mechanism of protein insertion into membranes. The last sixty-odd residues at the extreme C-terminus of the protein show many features reminiscent of signal peptides that target proteins to bacterial plasma membranes or to the endoplasmic reticulum of eukaryotic cells. These features include a long stretch of hydrophobic residues with a centrally located glycine (residue 166), followed by a charged C-terminus containing basic residues[17] (Fig. 1). The signal-like peptide adopts a predominantly α-helical structure in colicin A, which is in accord with circular dichroism results for many signal peptides. The hydrophobic part of the signal-like peptide is almost completely buried in the protein, which provides an energetically favourable situation in which a signal peptide can be transferred through a water-soluble medium. It is known that deletions in the regions of the surface helices of the peptide fragment result in precipitation of such mutants in the cytoplasm of the producing cell and thus impede colicin export[15]. It follows that the shielding of the hydrophobic region of signal peptides in the water-soluble medium of a cell is an important feature of transported proteins. The helical hairpin structure of the signal-like peptide supports earlier theories that helical hairpin motifs would be a major feature in models of spontaneous insertion of proteins into membranes[18]. The structure resolves the paradox of how a water-soluble protein can also be incorporated into membranes. Surfaces required to interact with the hydrophobic core of membranes are buried within the conformation of the water-soluble form of the protein: colicin A is an 'inside-out' membrane protein. This result is consistent with other recent experimental data indicating that proteins require a flexible conformation in translocating across membranes[19–24].

The features suggested as being necessary for insertion of colicin A into membranes seem to apply, with some additional requirements, to a wide variety of biological systems. Prime examples include the membrane-attack complex of the complement cascade[25], the action of various toxins[25] and insertion of membrane precursors[26]. Current work is directed towards determining the structure of the membrane-bound form of colicin A to understand further the mechanism of insertion and to explain the pore properties of the voltage-gated channel formed.

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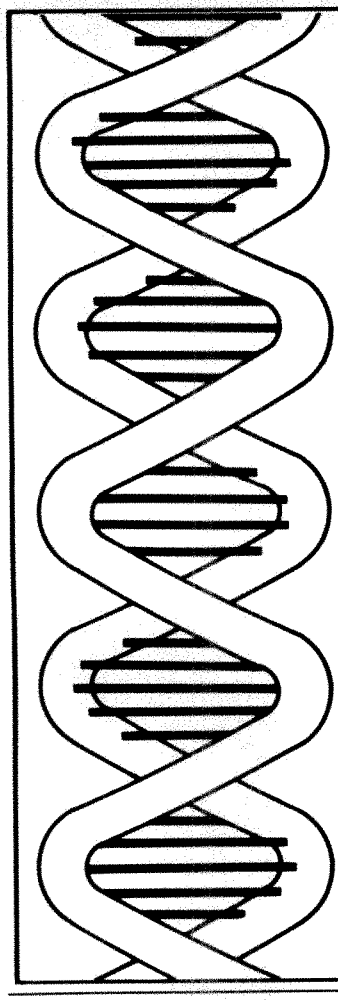
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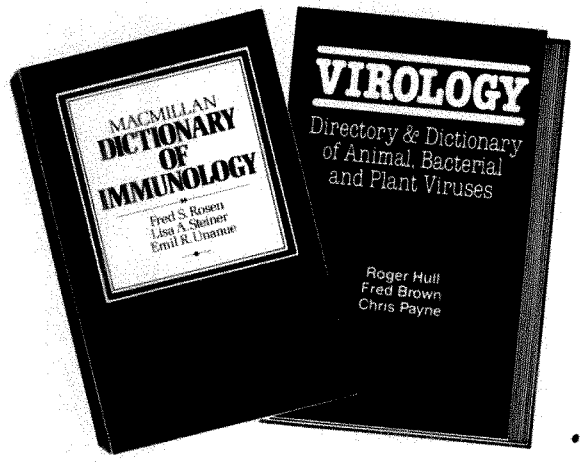
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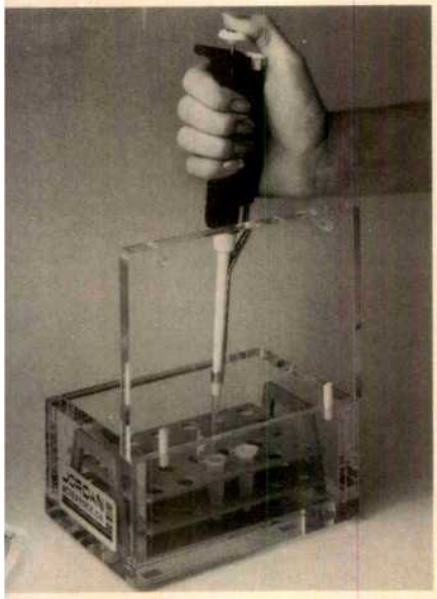
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New products for a new year

Start the year off with a new addition to the laboratory — this week's selection includes an infrared hotplate, a software model for teratogenicity studies, and a balance that calculates molarity.

Bio Medical Equipment offers what may well be the most inexpensive **electrophoresis apparatus** on the market: the Mac Gel electrophoresis kit (*Reader Service No. 100*). For a total price of $10 (US), the Mac Gel kit contains two gel-forming plates, a multiwell comb, and platinum electrodes. Bio Medical Equipment's kit prepares standard-sized gels, and the entire apparatus fits into a buffer tank with ample dimensions for large buffer volumes. It is possible to use the electrodes with virtually any controller.

From Jordan Scientific comes a microcentrifuge **tube rack/beta shield** with a unique two-position lid (*Reader Service No. 101*). With the lid down, the rack is



Storage and safety are the two objectives fulfilled by Jordan Scientific's tube box.

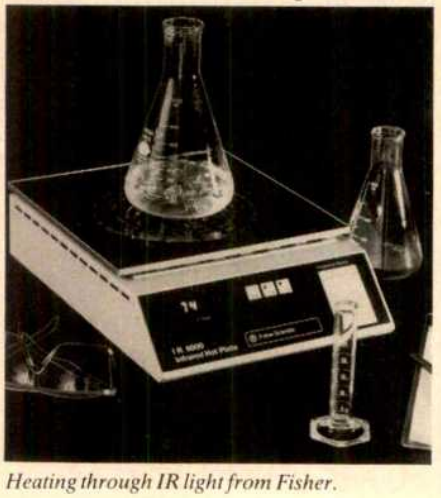
fully enclosed for the safe storage and transportation of tubes containing beta-emitting isotopes. In the upright position, the lid shields the user from radiation during pipetting operations or tube handling. The tube rack is made of one-half-inch-thick acrylic, and can withstand autoclaving. It holds 24 1.5-ml Eppendorf-type tubes in a four-by-six array, a configuration Jordan Scientific says is ideal for performing sequencing reactions. The tube rack costs $55 (US).

Lab essentials

The newest **spectrophotometer** from Pharmacia LKB is the Novaspec II, an instrument that the company terms "simplicity itself" (*Reader Service No. 102*). The compact Novaspec II performs absorbance and transmission measurements in only three steps: the required wavelength is set using the keypad, the reference blank is adjusted by one keystroke after inserting the blank cuvette, and the sample measurement is read off of the digital display. The instrument can make concentration measurements, perform simple kinetics calculations, and measure absorbance ratios and differences. It comes with a bi-directional RS232C interface for connection to a printer for hard-copy results. The Novaspec II's wavelength range is 325–900 nm, monitored by a single detector. It accepts both 10 mm cuvettes and standard-sized test tubes.

Sartorius has just announced the launch of a new series of **analytical balances** that can perform the necessary calculations for preparing molar solutions (*Reader Service No. 103*). The balances come equipped with Sartorius's Lab-Plus software, which has programs for statistical analyses, density determination, and classification and sorting operations. For preparing molar solutions, the operator keys in the molecular weight of the substance to be dissolved, the desired molarity and the target volume — the balance displays the amount of substance to be "filled toward zero". If the operator adds too much, the balance recalculates the target volume to attain the same molarity. The balances can weigh directly in moles, percent by volume or p.p.m. when provided the molecular weight and target volume. The capacity of the balances is 200 g, displayed down to 0.1 mg.

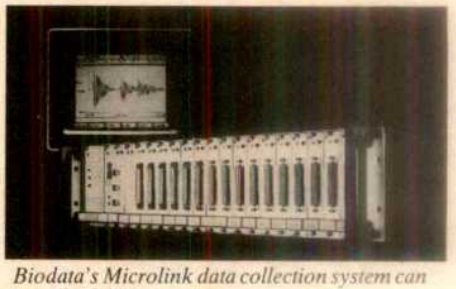
Fisher Scientific has a new **laboratory hotplate** that uses infrared light instead of an electrical heating unit, to provide a maximum temperature of 615 °C (*Reader Service No. 104*). The company says the hotplate can boil 1,000 ml of water in under 12 minutes in a 1.5-litre Erlenmyer flask — nearly twice the heating rate of most other hotplates. The $650 (US) IR-6000 hotplate is continuously adjustable over its power setting range of between 1 per cent and 99 per cent. Fisher Scientific says the hotplate assures greater uniformity of heating than traditional hotplates because it does not depend on the shape of the vessel bottom: the infrared light generated by the unit's molybdenum disilicide coil heats the sample directly. The glass-ceramic surface of the IR-6000 measures 12 × 12 inches, and its stainless steel edge can contain up to 400 ml of liquid in the event of a spill.



Heating through IR light from Fisher.

Computing

For those who collect large volumes of analogue data from laboratory instruments or transducers with voltage outputs, Biodata offers its Microlink high-speed **data collection system** (*Reader Service No. 105*). Biodata's system collects and streams data directly to the hard disk of an IBM microcomputer or compatible at rates of up to 25,000 samples per second. The data is available immediately for analysis, and the unit can store up to 16 million samples. The data acquisition hardware accepts up to 256 channels in one cabinet and incorporates 12-bit buffered analogue-to-digital conversion. Both voltage and thermocouple inputs can be used with the £4,000 (UK) Microlink system, which includes hardware, software and IBM-compatible computer.



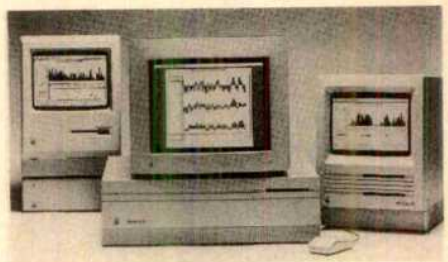
Biodata's Microlink data collection system can handle 25,000 samples per second.

The HDi corporation offers an alternative to animal testing for **teratogen analysis** — a set of advanced structure- activity (SAR) models of teratogenesis coupled with its Topkat software (*Reader Service No. 106*). HDi says the models and the Topkat program can predict the teratogenicity of untested chemicals with an

accuracy of 96 per cent. The models are based on a data base of teratogenesis bioassays that HDi abstracted from the literature. Independent parameters in the SAR equations include electronic charges, substructural keys and topological descriptors. The model is based on four subequations, but this is transparent to the user: Topkat automatically selects the equation appropriate for the chemical to be estimated. HDi says the $15,000 (US) system correctly classifies carbocyclic aromatics, heterocyclic aromatics, acyclic aliphatics and alicyclics over 95 per cent of the time. Topkat also has modules to predict the probability of carcinogenicity, mutagenicity, skin and eye irritation, and values of acute toxic effects.

An interactive **graphics software package** for exploring, analysing, and visualizing technical data is new from Precision Visuals (*Reader Service No. 107*). PV-WAVE stands for Precision Visuals' Workstation Analysis and Visualization Environment. It allows researchers to navigate through large datasets, reduce and filter the data into manageable subsets, visually distinguish and analyse key features and trends, and translate results into publication-quality charts, graphs, contour maps, surface plots and images. Commands can be entered using the keyboard or be grouped into procedure files; application developers can create custom systems using PV-WAVE's menu development tools. Running on a VAXstation 2000, Precision Visuals says the software can read 2,400 floating-point numbers in 1.6 seconds, or compute a 4,096-point real-to-complex Fast Fourier Transform in 0.15 seconds. PV-WAVE costs $3,350 for the VAXstation 2000. Sun workstation versions will be released early this year.

A desktop **DNA sequence analysis software package** for use with Macintosh computers has been introduced by International Biotechnologies, Inc. (*Reader Service No. 108*). The $2,295 (US) IBI Pustell Software for the Macintosh supports over 100 applications, including restriction analysis, homology matrices and protein structural studies. It also provides a global search function that matches DNA-DNA, protein-protein and protein-DNA with designated databases. The software interacts with and converts



DNA sequence analysis comes to the Macintosh computer with software from IBI.

to every major sequence format, says IBI, including that used in GenBank, Bionet, Staden, Line and UWGCG. A unique "wildcard" feature allows researchers to access data without complete reference information. IBI says researchers can use data from their Macintosh in a VAX or IBM-PC through a user-supplied interface, permitting users to share data with colleagues.

Analytical aids

Spectramass Ltd's BioQuad gas analysis system uses quadrupole mass spectrometry technology to **monitor fermentation** processes (*Reader Service No. 109*). The #15,000-19,000 (UK) system analyses inlet and exhaust gases, and provides data on dissolved gases and volatile substrates. The heart of the system is the Spectramass PC 2000 mass spectrometer, which has been designed with ion source optics especially suited for the analysis of complex gas streams. Spectramass says the analysis of dissolved gases and volatiles is easily accomplished using a membrane inlet system and probe to permit connection to standard fermenter ports. The BioQuad system includes vacuum components, remote-controlled valves and inlet lines, and software.

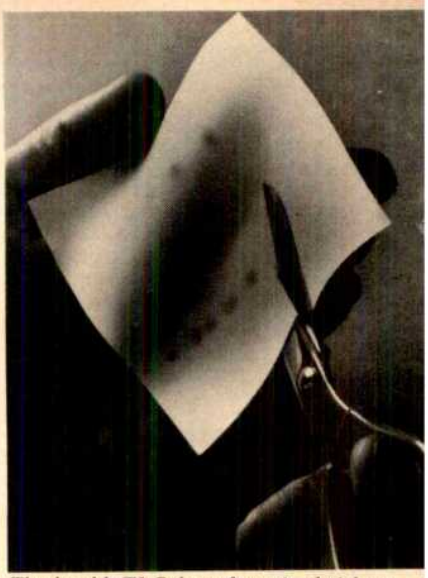
To protect HPLC equipment against damage from reagent backflow, Pickering Laboratories has designed its post-column **eluent flow interlock** (*Reader Service No.*



Prevent post-column eluent backflow with the interlock device from Pickering Labs.

110). The interlock system monitors pressure at the outlet of the eluent pump, and shuts off the power to the post-column system if the eluent pressure drops below 500 p.s.i., stopping reverse reagent flow that can damage both the analytical column and post-column equipment. Pickering Labs' interlock also shuts down the post-column system in the event of power loss, and a surge protection feature guards against electrical damage from voltage spikes. The $420 (US) interlock comes equipped with four 120-V receptacles, stats lights, reset button, circuit breaker, power cord and a pressure switch with a five-foot cord.

Analytichem has introduced a **thin-layer chromatography sheet** that it says is so flexible that spots can be cut or punched



The durable TLC sheets from Analytichem won't crumble during handling.

out for further analysis without resortin to tedious scraping (*Reader Service N 111*). Analytichem's Empore TLC shee were developed with the 3M corporatio and are composed of inert PTFE fibr with chemically bonded silica particle Because the sheets are produced without binder, Analytichem says they can be use in a wide range of applications with opt mum resolution and reproducibility. Th company also says the Empore TL sheets exhibit several times the samp capacity of a standard hard-layer TL plate, preventing problems with ove loading and spot distortion. The 2.5 10 cm and 10 × 10 cm sheets are availab with a variety of bonded silicas.

Enzymes and reagents

Janssen Life Sciences Products has a ne ultra-small **immunogold probe** for incre sed labelling efficiency and penetratic power in the detection of antigens ar biotinylated probes using the immunogo silver staining technique (*Reader Servi No. 112*). The AuroProbe One reage contains colloidal gold particles measu ing 1 nm, and is designed so that the rat between probe protein and gold particle less than one. Because AuroProbe O can be used in a variety of applicatio from immunoblotting to electron micr scopy, it allows results from differe methods to be compared. The signal pr duced by AuroProbe One can be visu ized by silver enhancement usi Janssen's IntenSE reagents for micr scopy and blotting experiments. Ea package of AuroProbe One contains 1 AuroProbe One reagent, 10 ml IGS quality gelatin and detailed instructions

Amersham has recently introduced Multiprime system for **rapid hybridizati** reactions (*Reader Service No. 113*). T new kit is based on Amersham's Mul prime random priming labelling kit, a

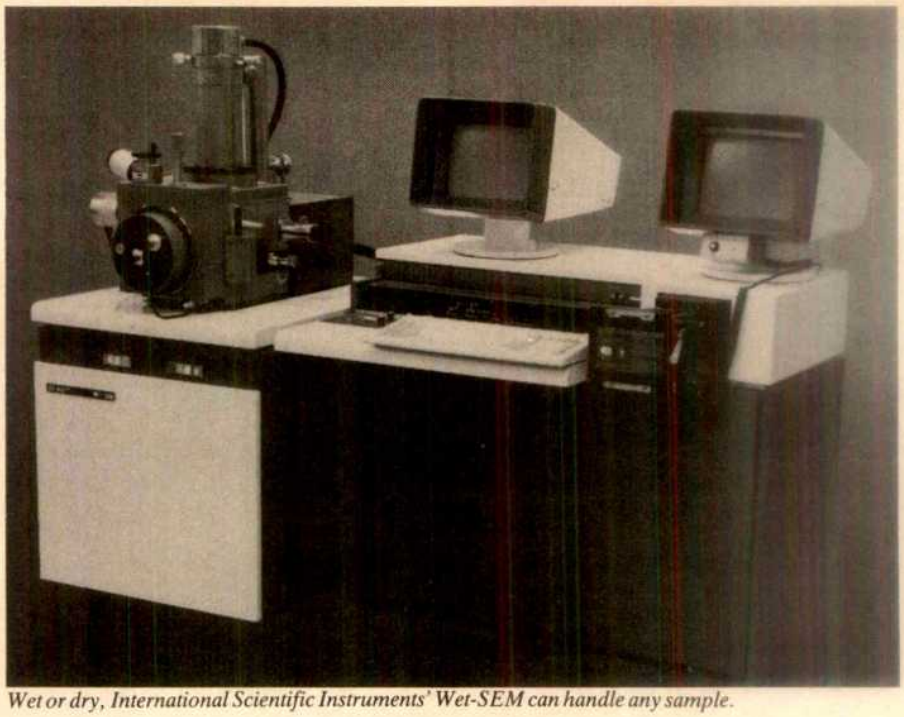
allows the synthesis of high specific-activity probes in 30 minutes, and subsequent hybridization with nucleic acid blots in two hours, says the company. The system consists of a DNA labelling kit and a rapid hybridization buffer that increases the hybridization rate without requiring high probe concentrations. Amersham says when using a probe concentration of between 2 and 8 ng nl^{-1}, the sensitivities obtained after a two-hour hybridization are equivalent or higher than sensitivities obtained in an overnight incubation using conventional buffers, without an increase in background.

GIBCO Laboratories has two new **serums** that it says provide an economical and more readily available alternative to fetal bovine serum (*Reader Service No. 114*). GIBCO's supplemented calf serum and calf supreme serum contain defined growth-promoting additives, including vitamins, trace elements, transferrin, insulin, and proprietary growth factors. GIBCO recommends its supplemented calf serum for researchers who experience lot-to-lot variability when using calf serum, and those for whom a high-performance basal calf serum would suffice. The company reports developmental studies indicate that a five per cent supplementation of calf serum in basal medium yields superior results over calf serum, and approaches results with fetal bovine serum. GIBCO says a five per cent supplementation of calf supreme serum in basal medium yields results comparable to fetal bovine serum.

A new version of the Sequenase **DNA polymerase** from United States Biochemical Corporation is now available (*Reader Service No. 115*). Version 2.0 of Sequenase was developed by *in vitro* genetic manipulation of the gene for T7 DNA polymerase which eliminated the exonuclease activity of the enzyme, giving it properties suitable for dideoxy DNA sequencing. US Biochemical says the new version retains all the advantages of the original Sequenase — low gel backgrounds, uniform band intensities, resolution of 600 or more bases, and the ability to use d1TP to remove gel compressions — but also demonstrates increased processivity and reduced pausing at regions of secondary structure. Sequenase Version 2.0 is available in 200- and 1,000-unit packages costing $90 and $355 (US), respectively, or as part of a kit with sufficient reagents for 100 sequences for $185 (US).

Microscopy

International Scientific Instruments has just announced the availability of a **scanning electron microscope** that can be used to examine wet, unprepared samples (*Reader Service No. 116*). ISI's Wet-SEM



Wet or dry, International Scientific Instruments' Wet-SEM can handle any sample.

eliminates the need for sample preparation, such as coating, fixing, dehydrating or critical-point drying. Because the microscope operates in a low-vacuum environment, the $72,500 (US) Wet-SEM can analyse moist specimens or even liquids. The uses of the Wet-SEM span a broad range of applications: it can be used with an optional EDX detector for elemental analysis, or can be switched to a high-vacuum setting for 6-nm resolution. Special features include an automatic focus system and an auto stigmator.

The Electron Microscope Supplies Division of EMCorp has a new **carbon-coater** that it says can deposit a thin film of carbon on specimens for electron microscopy in under two minutes (*Reader Service No. 117*). EMCorp's $3,650 (US) Hummer CDS II comes ready to use right out of the box. It can coat specimens of up to 3.25 inches in diameter, or 12 microscope stubs in a single procedure. Its two-stage direct-drive rotary vacuum pump delivers at least 20 mtorr absolute pressure, and its manual bleed valve introduces argon for processing at 80–100 mtorr. The coater automatically vents once the main power switch is turned off to prevent backstreaming. The Hummer CDS II uses a pre-sharpened dual carbon rod system that EMCorp says yields consistent coating. Particle turbulence in the chamber is prevented by a regulated vent system.



Carbon coating of EM samples in under two minutes by EMCorp.

Leitz Instruments Ltd is now supplying the Narshige range of **micromanipulators and ancillary equipment** with its Labovert and Fluovert inverted microscopes, and its Wild stereomicroscopes (*Reader Service No. 116*). Narshige's pipette puller, microforge, manual and automatic microinjectors, and micro beveller can now be purchased along with Leitz microscopes as a complete micromanipulation workstation which can be configured to suit a wide variety of applications.

Zeiss is bringing the line of **rotary microtomes** made by the German company Microm to the US market (*Reader Service No. 119*). Microm microtomes come in designs for routine paraffin histology, advanced plastics histology and industrial microtomy. Zeiss says the Microm microtomes have backlash-free and maintenance-free roller bearing guideways, enabling them to achieve high precision and stability. Specimen slices between 0.5 µm and 60 µm in thickness are routinely achieved, according to the company. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

What prospects for the older graduate?

Richard Pearson

Employers have been slow to adjust to the changes in graduate supply. Soon, with decreasing numbers of school leavers, they may have to.

LAST year was the year of the school leaver, or rather concern about the lack of them in the 1990s: as the number of school leavers in the United Kingdom will have declined by 35 per cent over the decade to 1994. With the economy booming and unemployment falling, a shortage of young people will affect employers, trainers and higher education alike (*Nature* **335**, 100; 1988). In the case of higher education the hope is that more mature students can be found who, along with other under-represented groups such as women, ethnic minorities and those from the lower social classes, will, along with a rising participation rate among young people, fill the empty places in higher education and boost the numbers graduating to meet the needs of the economy.

Since the late 1970s there has been growing interest in improving mature students' access to higher education, with more than 400 special access courses being launched to help mature students who lack the standard qualifications for access to higher education. At the same time most institutions now offer applicants aged over 21 selection criteria that do not rely on the normal academic qualifications. Just over half the mature entrants to full-time degree courses lack the traditional entry qualifications, rising to 75 per cent for those entering part-time courses.

Mature numbers

During the past decade the number of mature entrants in higher education has increased by over 50 per cent to total over 186,000 in 1986, driven mainly by the growing number of women and those on part-time courses, the number of men entering full-time university courses rising by only 10 per cent. The overall proportion (aged 21 or over at the time of entry) has now reached over 11 per cent in the universities and 34 per cent in the polytechnics and colleges. The majority of mature students are, however, studying at sub-degree level and on a part-time basis. These older students also have rather different subject preferences to their younger counterparts, with a much higher representation in the humanities and the social sciences, and lower representation in the sciences, technology and engineering.

In addition there is the Open University which was established to meet the needs of the mature, part-time student, many of whom are already in employment. Some 8,000 students graduate each year from the Open University, and of new entrants over 60 per cent are aged 30 or over.

It is worth noting that while in the United Kingdom the mature first degree graduate is still relatively rare, the majority being aged 21–22, this contrasts with the pattern in mainland Europe where courses take longer and many graduates only qualify in their mid to late 20s.

In trying to achieve a further increase in the number of mature entrants, institutions will be facing growing competition from employers for the good mature entrant as both they and higher education seek to make up for the shortfall of school leavers. One important determinant of who wins this battle will be students' responses to loans and the way they trade cash in the hand for potentially higher earnings later in life. One concern, expressed even before the loans proposals were introduced, is the financial barrier facing students taking access courses who were not eligible for mandatory grants. A recent survey of students suggested that half of them would not have started their access courses if the grant had not been available.

What then are the prospects for mature graduates, and will they benefit alongside the traditional young graduate in the booming graduate labour market of the 1990s (*Nature* **334**, 90; 1988)? Looking back to the mid-1980s, when the graduate market was starting to take off, mature graduates were slightly less likely to be in employment and also less likely to be unemployed, with a higher percentage going into further education and training or not being available to work. The latter figure is rather higher still for mature women than for mature men.

When due allowance is made for subject differences mature graduates had rather better employment rates in education, with little difference being apparent in the case of engineering. In the case of the sciences, administration and business studies, and humanities, they fared rather worse, with both lower employment and higher unemployment rates.

How then are mature entrants treated in the labour market? One problem they face is that many employers, especially in the private sector, structure their recruitment, training and career development programme around the 21 year olds, seeking to get them into management positions by their late 20s. A recent survey has, however, shown that while half of the respondents say they treat all their applicants equally, one in three expressed reservations about applications from mature graduates, with one in five specifying age limits, normally 30 or under, in their recruitment literature. Teaching is a notable exception where positive efforts are being made to attract mature entrants.

Most welcome

More generally the most welcomed were those applicants who had a relevant degree and relevant work experiences, although there was still an age threshold of about 30 after which entry could become more difficult. Much depends on the relevance of the work experience and the job applied for, it being much harder to enter a general training scheme than entering a direct appointment. For those with a non-relevant degree the opportunities were more restricted, reflecting in part the more limited opportunities open to this group of conventional graduates. In general, difficulties increased with age and with the decreasing relevance of qualifications.

The main perceived disadvantages of the mature graduate were their apparent poor mobility, poor adaptability, too high salary expectations, difficulties fitting in with younger peers and not fitting into promotion structures. Given that many companies do not recruit them, it must be assumed that they have a strongly stereotyped picture of them. On the positive side their maturity, stability, commitment and better social and interpersonal skills were often highlighted. Another positive sign for those in their mid-20s is recruiters growing interest in 'second bounce' graduates, those who re-enter the job market quickly after a mistaken first choice.

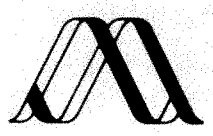
With the market expected to tighten further into the 1990s only a minority of recruiters when surveyed say they are looking to adjust their policies, despite the fact that mature graduates will be an increasing proportion of those graduating in the future. As with school leavers, most recruiters seem set to carry on with policies appropriate to a time of abundance in the labour market rather than one of stiffening competition. For the mature graduate, economic conditions and skill shortages are going to be the fastest way of breaking down traditional stereotypes.

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton BN1 9RF, UK.

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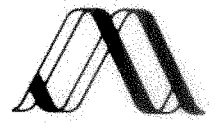
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POSTDOCTORAL POSITION

available from April 1989 for a minimum of 2 years to study *in vitro* synaptic physiology and lesion-induced neural plasticity of the vestibular system of lower vertebrates. A background in cellular electrophysiology is required while experience in intracellular recording, in neuropharmacological or in anatomical techniques would be desirable.

Applicants should send a Curriculum Vitae together with letters of recommendation before March 1, 1989 to: **Dr. N. Dieringer, Department of Physiology, Pettenkoferstr. 12, 8000 München 2, West Germany.** (W5721)A

ASSISTANT PROFESSOR (RESEARCH SCHOLAR)

Faculty of Medicine, Department of Physiology, University of Alberta
$32,564–$46,700

Applications are invited for the position of Assistant Professor (Research Scholar) in the area of Renal Physiology. Qualifications include a Ph.D. or M.D. degree and a demonstrated outstanding ability for independent research. The successful candidate will participate in the department's teaching program (both graduate and undergraduate) and other academic activities. The position will depend upon funding through an external agency such as The Alberta Heritage Foundation for Medical Research, and the effective date of employment is dependent upon the success of the application.

In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

Interested applicants should submit their Curriculum vitae, an outline of proposed research, and the names of three referees by January 31, 1989, to:

Dr. P.K.T. Pang, Professor & Chairman, Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7

The University of Alberta is committed to the principle of equity in employment. (NW3049)A

DEVELOPMENTAL BIOLOGIST MCGILL UNIVERSITY

The Department of Biology invites applications for a tenure-track position at the Assistant Professor level, effective September 1, 1989. We seek a developmental biologist using cellular, molecular and/or genetic approaches. The successful candidate is expected to establish a strong research program and to participate in teaching developmental biology.

Send curriculum vitae, statement of research interests and goals, representative reprints, and three letters of reference to **Dr. Barid Mukherjee, Chairman, Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada H3A 1B1.** Deadline: February 15, 1989. (NW3048)A

Sandoz Institute for Medical Research

Laboratory Head in Molecular Biology

The Sandoz Institute is seeking an experienced molecular biologist with a good knowledge of cloning methods, preferably in the areas of expression cloning, gene transfer methods and site-directed mutagenesis. The person appointed, who will have at least 3 years' post-doctoral experience, will be in charge of a small group, and will also be expected to play an active role in the overall activities of the Institute, contributing to its long-term drug development goals. He or she should have broad interests in molecular neurobiology, so as to be able to make good use of the collaborative opportunities that the Institute offers. Initially, the emphasis will be on the cloning and characterisation of the bradykinin receptor.

The Sandoz Institute, which opened in 1985, is a multidisciplinary research organization comprising about 60 staff (45 scientific and technical staff) working on a range of neurobiological projects which have as their long-term objective the discovery of new types of analgesic drugs. The Institute is housed in its own premises within University College London, and has strong academic links with the College, though it is administratively and financially independent. The laboratories are modern and well-appointed, and the Institute is well funded by Sandoz Ltd.

The starting salary will be in the range £25,000-£30,000 p.a., and is subject to annual review. A company car, pension scheme, medical insurance, generous holiday allowance and other benefits are included.

Applications, including a full *curriculum vitae* and the names of three referees, should be sent to the Director, Dr. H. P. Rang FRS, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, UK, Tel: 01-387 4445, by 31 January 1989. Candidates will be welcome to visit the Institute for a preliminary discussion if they wish.

(7992)A

Dyno Particles A.S is a small, expansive high-tech firm with a pronounced pioneering spirit and great international ambitions. The company is a wholly-owned subsidiary of Dyno Industrier A.S., one of Norway's largest chemical companies. The business consists of R&D, production and marketing of products based on Dynospheres®, monodisperse polymer particles. It is our goal to be able to offer the best products of their kind to the world market. Dyno Particles is currently concentrating efforts on the development of products for the chromatographic separation of bio-molecules. In addition, products based on Dynospheres® are presently used in a number of medical and technical applications. The firm is headquartered in Lillestrøm, just outside Oslo. An R&D department is currently being established in the Knivsta-Ar area, between Stockholm and Uppsala.

DYNO PARTICLES IS EXPANDING

In order to reach the high international goals we have set, we seek several competent employees. Persons willing to do their best are invited to join our exciting environment where the challenges are many and the possibilities are great. We place emphasis on cooperation and team spirit as the basis for progress and growth. Therefore, our new employees must have, in addition to in-depth technical expertise, a sense of flexibility and a well-developed ability to work with others.

SECTOR SEPARATION TECHNOLOGY

Sector Separation Technology is responsible for the development and marketing of products for the separation of complex (bio-)chemical mixtures. Our top-priority project involves developing liquid chromatography packings for use in biotechnology-R&D laboratories and the pharmaceutical industry.

The **Marketing Departments** within the Sector have responsibility for marketing and technical service.

The Departments will have great freedom to organize themselves such that the business areas will be as functional and effective as possible. During the current implementation phase, the group will have a strong core-team character. Our products will be of the highest quality and extremely stimulating to work with.

The home base for the business area will be Lillestrøm, but the work will be of a decidely international character involving a great deal of travel. Well-qualified people will be offered highly-competitive salary packages.

Marketing Strategist/Market Researcher

This position will be one of the staff functions and will involve working closely with the Sector's Vice President.

The applicant should have experience in industrial R&D and marketing. Alternatively, the applicant may have experience from a successful industrial new business development. Very strong expertise in the field of biochemical separation technology as well as understanding of the mechanisms behind successful new-technology market introductions are a requirement.

We seek a well-structured candidate with razor-sharp analytical abilities in combination with an open-minded attitude for new technology, ideas and ways of thinking.

Our new Marketing Strategist will be part of a group consisting of the Sector's top R&D and strategic management.

The application is to be marked "DP-1".

Marketing Managers

We are looking for **two Marketing Managers** who will have responsibility for marketing, sales support, and technical service within their respective areas. The applicants should have several years experience in marketing in the separation technology field or associated areas within biotechnology. We assume in-depth background in international marketing as well as a thorough understanding of separation technology.

The new Marketing Managers are expected to be very enterprising persons with the ability to motivate their employees. Being able to work well with others is important because we will be working in groups where open-mindedness and sincerity will be our central principles.

Extremely good working knowledge of English is a requirement; proficiency in other major languages will be a plus.

Marketing Manager – Laboratory Products

In additon to the above qualifications, the new Marketing Manager within this product area should have an R&D background, as well as experience in marketing high-tech laboratory products. A further requirement is a deep insight into the thought processes used in R&D and analytical laboratories, as well as within such divergent environments as universities, R&D institutions, government bodies and industry.

Please mark this application "DP-2".

Marketing Manager – Industrial Products

In addition to the above joint qualifications, the new industrial products Marketing Manager should have good knowledge of, as well as many personal contacts within, the biotechnology or pharmaceuticals industry or both. Direct experience in industrial chromatography will be a plus.

This application is to be marked "DP-3".

Technical Service

For our Department for Industrial Products Marketing, we seek an employee to manage the Technical Service function.

The applicant should have a solid background in applied separation technology, preferably in industrial chromatography of bio-molecules, as well as experience in technical service within the biotechnology or pharmaceutical industry. It will be an advantage to have insight into industrial production and marketing.

Interest in working in close cooperation with the customers, as well as the ability to understand and solve their problems, are of great importance.

The responsibilities will also include arranging training programs for sales personnel and customers. The work will proceed on an international basis and good language abilities, foremost English, will therefore be necessary.

Please mark the application "DP-4".

For more information concerning these positions please contact Kristen Fretheim, 47-2-31-76-81 (Norway), or, Lennart Söderberg, 47-2-31-75-24 (Norway) or 46-18-32-30-62 (Sweden).

Please deliver your application by 26th January to DYNO INDUSTRIER A.S., Chemicals Group, P.O. Box 160, N-2001 Lillestrøm, Norway.

DYNO

The Wellcome Trust

Scientific Officer

The Wellcome Trust wishes to appoint a scientific officer to assist Dr. L. H. Smaje, Assistant Director in the administration of the Trust's programme in Physiology and Pharmacology.

This permanent position will involve processing grant applications, production of background policy papers and interfacing between the scientific community and the Trust.

The salary will initially be within the range £17,611 - £21,701. (Starting salary will be dependent upon age and experience.) Other employment conditions, including a non-contributory pension scheme, are excellent.

Candidates with several years' post doctoral research experience and a broad interest in science and medicine are invited to send a **curriculum vitae** and covering letter explaining why they are interested in a career in research administration to:

Deirdre Carty, The Wellcome Trust, 1 Park Square West, London NW1 4LG. Tel: 01-486 4902.

Informal enquiries are welcome and potential applicants are invited to telephone Dr. Smaje on the above number.

The closing date for applications is 18th February 1989.

(8004)A

ACADEMIC GASTRO-ENTEROLOGIST/ HEPATOLOGIST

to join a new investigative unit. Applicant must have strong background in basic research (immunology, cell biology, virology or liver injury desirable). Level of academic appointment is based on experience and qualifications. **Dr. George B. McDonald, Division of Clinical Research (M246), Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104.** An equal opportunity/affirmative action employer. (NW3057)A

Post Doctoral Fellow/ Research Associate

To examine the roll of Protein Kinase C in mediating the growth and differentiation of hematopoietic cells. Studies will focus on the control of transcription by the interaction of PK-C with the jun oncogene. Please send CV and names of three refs. to **Dr. A. Kraft, Div. of Hem/Onc, Univ. of Alabama at B'ham, 513 THT, 1900 Univ. Blvd., B'ham, AL 35294.** (NW3071)A

PLANT MOLECULAR BIOLOGY, TOULOUSE — FRANCE

GROUP LEADERS

The Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes CNRS-INRA in Toulouse is seeking to recruit two Group Leaders. The laboratory is located close to the Toulouse University campus, the second largest in France for Science and Technology. At present there are nine groups working on the molecular genetics either of the *Rhizobium*-legume symbiosis or of plant diseases caused by bacteria or viruses. The laboratory is to be enlarged in 1989 and two permanent positions for senior scientists will be created.

The appointees will have to develop groups working on plant genes involved in plant-microbe interactions. Excellent scientific credentials and at least four years of post-doctoral research experience in cellular molecular biology are required.

Annual salary will be in the range FF 150,000-200,000 depending on qualification and experience.

Further information may be obtained from **Dr. Georges Truchet, Laboratoire de Biologie Moléculaire, CNRS-INRA, BP27, 31326 Castanet-Tolosan Cedex, France,** to whom applications including a CV and the names of 3 referees should be sent. Closing date 28 February, 1989. (W5708)A

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF BIOCHEMISTRY AND GENETICS

POSTDOCTORAL RESEARCH ASSOCIATESHIPS IN MOLECULAR BIOLOGY

Applications are invited for three postdoctoral positions:

(1) Location of antibody neutralisation sites in Newcastle disease virus. This work, currently in progress, involves sequence analysis of monoclonal antibody resistant mutants with a view to the development of an improved vaccine against fowl pest. (Supported by the Agricultural and Food Research Council until February 16 1990).

(2) Expression, purification and characterisation of the RNA dependent RNA polymerase of Newcastle disease virus. This 250 KDa protein is present in only low amounts in the virus and infected cells. We have cloned and sequenced the gene and propose to overexpress it and purify the enzyme. We then propose to study the mechanism of action of the pure enzyme in replication and transcription *in vitro*. (Supported by a new three-year grant from the Wellcome Trust.

(3) The RecBCD protein of *E. coli*: role of the RecD subunit. We have previously cloned and sequenced the *recB, recC* and *recD* genes which are involved in DNA repair and genetic recombination. We are presently investigating the interaction of purified RecB, RecC, RecD and RecBCD proteins on model DNA substrates *in vitro*. (Supported by a new three-year grant from the Medical Research Council).

Candidates for all positions should ideally have experience of recombinant DNA techniques. Additional experience with tissue culture techniques would be advantageous for project (1) and preparative enzymology for projects (2) and (3). Starting salary will be up to £11,680 (plus superannuation) on the 1A scale.

Applications including a full *curriculum vitae* and the names and addresses of two academic referees should be sent to **Prof. Peter T. Emmerson, Department of Biochemistry and Genetics, The Medical School, University of Newcastle upon Tyne, NE2 4HH, UK.** (7996)A

FACULTY POSITION IN PROKARYOTIC GENETICS

The Department of Biology at Texas A&M University invites applications for a tenure-track position in bacterial or bacteriophage genetics and molecular biology. Candidates should be using a prokaryotic organism to study fundamental biological problems. Special consideration will be given to applicants interested in developmental, regulatory or membrane systems. Essential qualifications include a strong record of research accomplishment, the ability to develop a vigorous research program, and a commitment to training undergraduate and graduate students. The Department has excellent research facilities and will provide competitive salaries and startup funds. Please send curriculum vitae, a statement of research interests, and the names, addresses and telephone numbers of at least three references by February 15, 1989, to:

Dr. Michael D. Manson
Chairman, Microbiology Search
Department of Biology
Texas A&M University
College Station, TX 77843

Texas A&M University is an Affirmative Action/Equal Opportunity Employer. Minority candidates are especially encouraged to apply.

(NW3061)A

UBC

The University of British Columbia

Biotechnology Laboratory

Fermentation, Process Engineering and Bio-Instrumentation

The University of British Columbia has recently initiated the Biotechnology Laboratory under the directorship of Dr. Michael Smith. Its objective is to foster an interactive approach to problems in biotechnology. A total of ten new tenure track positions are being filled during 1987-89. Appointments will be primarily at the rank of Assistant Professor. Applicants must have a Ph.D. degree. Post-doctoral experience is an asset. Successful candidates will be expected to develop their own vigorous research programs and to collaborate with other faculty members on problems in one of the following areas: human, animal, plant and forest biology and in fermentation and process engineering and bio-instrumentation (with special emphasis on mammalian cell culture and the production of specific proteins) and in the application of genetic engineering techniques to these problems. Substantial start-up funding will be provided. Faculty appointed to the Biotechnology Laboratory will have a joint appointment in an appropriate academic Department and will teach and supervise undergraduate and/or graduate students in that Department's programs or in academic programs to be established under the aegis of the Biotechnology Laboratory. At this time, candidates are sought with research expertise in the following area:

Bio-Instrumentation *(1 position)*. Monitoring and control of bioreactors and purification processes. Development of biosensors and measuring circuits. The applicant should have the ability to collaborate with biochemistry and electrical engineering faculty members and students, and to develop a teaching program bridging these disciplines.

Process Engineering *(1 position)*. Development of upstream and/or downstream processing techniques relevant to the purification of biologicals. The applicant should be experienced in the application of genetic engineering principles, in modern purification procedures for complex biomolecules and be capable of developing a teaching program bridging the areas of genetic and process engineering.

Appointments will be made starting 1st July 1989 or as soon as possible thereafter. Closing date for applications, 1st February 1989. Salary will be commensurate with qualifications and experience. The University of British Columbia offers equal opportunity for employment to qualified female and male applicants. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

Applications, including a full c.v., plans for future research (approximately 2-3 pages), and the names, addresses and phone numbers of three references, should be sent to: **Mrs. Darlene Crowe, Administration, Biotechnology Laboratory, The University of British Columbia, Room 237 - 6174 University Boulevard, Vancouver, B.C., Canada V6T 1W5.**

NW(2887)A

POSITIONS AVAILABLE 1988-89

The School for Field Studies seeks Senior and Associate faculty for semester and summer programs in the following specialties: Tropical Species Diversity/Australia; Ethnobotany/Ecuador; Landscape Ecology/Colorado; Tropical Ecology/Costa Rica; Acid Rain/Adirondacks; Wildlife Management/Kenya; Cultural Ecology/Himalayas; Bald Eagles/Alaska; Coral Reef Ecology & Marine Parks Management/Virgin Islands; Monkseals/Greece; Endangered Monkeys/Mexico; Spinner Dolphins/Hawaii; Humpback Whale Ecology/North Atlantic; Bottlenose Dolphins/Alabama. SFS also seeks applicants for administrator/teachers to direct permanent Centers for Rainforest Studies/Australia; Marine Ecosystem Studies/Virgin Islands (coral reefs); Wildlife Management Studies/Kenya (big game ecology); and Marine Mammal Studies/Baja, Mexico (whale ecology).

QUALIFICATIONS: excellence in teaching; advanced training in environmental sciences/conservation (Ph.D for Senior Faculty, Master's degree for Associate Faculty; leadership and facility with group dynamics; commitment to experimental education; experience in field research situations; teamwork; and administrative ability (for Field Directors). Please submit cv and letter of interest and qualifications to the **Dean, School for Field Studies, 16 Broadway, Beverley, MA 01915 (phone (508) 927-7777; FAX (508) 927-5127)).** (NW3066)A

UBC

The University of British Columbia

Biotechnology Laboratory

(Human and Animal Biology)

The University of British Columbia has recently initiated the Biotechnology Laboratory under the directorship of Dr. Michael Smith. Its objective is to foster an interactive approach to problems in biotechnology. A total of ten new tenure track positions are being filled during 1987-89. Appointments will be primarily at the rank of Assistant Professor. Applicants must have a Ph.D. degree. Post-doctoral experience is required. Successful candidates will be expected to develop their own vigorous research programs and to collaborate with other faculty members on problems in one of the following areas: human, animal, plant and forest biology and in fermentation and process engineering and bio-instrumentation (with special emphasis on mammalian cell culture and the production of specific proteins) and in the application of genetic engineering techniques to these problems. Substantial start-up funding will be provided. Faculty appointed to the Biotechnology Laboratory will have a joint appointment in an appropriate academic Department and will teach and supervise undergraduate and/or graduate students in that Department's programs or in academic programs to be established under the aegis of the Biotechnology Laboratory. At this time, candidates are sought with research expertise in the following area:

Human and Animal Biology *(1 position)*. Research interests in the molecular genetics of mammalian development. In addition, the candidate should be interested in initiating research interactions with other faculty members of the University who study animal and/or human development.

Appointments will be made starting 1st July 1989 or as soon as possible thereafter. Closing date for applications, 1st February 1989. Salary will be commensurate with qualifications and experience. The University of British Columbia offers equal opportunity for employment to qualified female and male applicants. In accordance with Canadian immigration requirements this advertisement is directed to Canadian citizens and permanent residents.

Applications, including a full c.v., plans for future research (approximately 2-3 pages), and the names, addresses and phone numbers of three references, should be sent to: **Mrs. Darlene Crowe, Administration, Biotechnology Laboratory, The University of British Columbia, Room 237 - 6174 University Boulevard, Vancouver, B.C., Canada V6T 1W5.**

NW(2888)A

Cird
Centre International de Recherches Dermatologiques

SOPHIA ANTIPOLIS – VALBONNE

RECRUTE

UN CHERCHEUR EN PHARMACOCINETIQUE ET METABOLISME (H, F) DOCTORAT, Ph.D. ou équivalent

Le CIRD est un Centre de Recherches International, situé dans le Sud-Est de la France, qui a pour vocation la recherche de nouveaux produits destinés au traitement des maladies de la peau.

Le poste sera rattaché au département de Biopharmacie, dans le groupe de pharmacocinétique et métabolisme. Son titulaire aura pour fonction principale:

la responsabilité de ses travaux de recherche, qui seront plus particulièrement orientés vers le domaine de l'identification des métabolites de nouveaux produits pharmaceutiques.
Il sera amené à utiliser des techniques telles que spectrométrie de masse et HPLC.

Il sera par ailleurs impliqué dans l'ensemble des activités du groupe.

Le candidat doit avoir une bonne connaissance pratique des méthodes avancées, d'analyse des produits dans les milieux biologiques, et d'identification des métabolites.

Une à deux années d'expérience post-doctorale sont souhaitées.

Ce poste demande une réelle capacité de communication et d'interaction avec les membres du groupe et les scientifiques du Centre.

Il implique par ailleurs une réflexion et une démarche intégrant les règles des "bonnes pratiques de laboratoire".

ADRESSER C.V. + LETTRE MANUSCRITE + PHOTO (sous référence 88-10/07) AU SERVICE DU PERSONNEL – C.I.R.D. – SOPHIA ANTIPOLIS – ROUTE DES LUCIOLES – 06560 VALBONNE FRANCE. (W5720)A

CHIEF SCIENTIFIC OFFICER

The Imperial Cancer Research Fund (ICRF) Laboratories in the Institute of Molecular Medicine, John Radcliffe Infirmary, Oxford are due to open in the spring in 1989.

The fund is seeking a Chief Scientific Officer to coordinate and run the technical support services for the laboratories.

The ICRF Laboratories will initially occupy 7,000 sq. ft. with expansion to 9,000 sq. ft. in the future. They will be largely self sufficient with common instrument rooms and service areas. There will be a number of independent research groups with differing but related interests. The number of staff will be approximately 18 in the first instance, increasing to 40 in 1989, and 55 in 1990 to a maximum of around 70 at full complement.

As a member of the Central Research Division, the Chief Scientific Officer will be reponsible for the day to day administration of the laboratory services.

Applicants should be qualified to degree level (or equivalent) in a scientific subject and should possess substantial experience of laboratory administration. Ability to organise own work and act with a degree of independence. Age 30+.

Salary range £14,584 to £17,145.

For further information and application form please write or telephone Mrs P. Harwood, Recruitment Officer, Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, on 01-242 0200 ext. 2357 quoting ref 45/R.

Smoking is actively discouraged. (8024)A

IMPERIAL CANCER RESEARCH FUND

KING'S COLLEGE LONDON
Department of Biochemistry

Two Postgraduate/Postgraduate Research Positions (Chemists/Biochemists/Pharmacologists)

1) THE ROLE OF OXIDANTS IN HUMAN ATHEROSCLEROSIS

Applications are invited for the position of post-doctoral scientist to participate in a study of the role of oxidants in human atherosclerosis (two years in the first instance). The work will be based in the laboratory of Professor B Halliwell, but occasional trips to Cambridge will be necessary to collect human clinical material. Candidates should have a first degree and a PhD in Chemistry, Biochemistry or Pharmacology. Good experience of analytical methods, particularly HPLC and GC-mass spectometry, would be advantageous. Salary in range £9,865-£12,150 plus London Weighting (currently £1,650).

Applications including a full CV and the names of two referees should be sent as soon as possible to **Professor B Halliwell, Department of Biochemistry, King's College London, Strand, London, WC2R 2LS, UK.**

2) THE ROLE OF NEUROTOXINS IN PARKINSON'S DISEASE

This position is supported by the Parkinson's Disease Society (two years in the first instance) and is concerned with potentially neurotoxic natural products. Good experience in analytical methods, particularly HPLC, would be advantageous. Salary in the range £10,315-£11,515 including London Weighting.

Applicants including a full CV and the names of two referees should be sent as soon as possible to **Dr P B Nunn, Department of Biochemistry, King's College London, Strand, London WC2R 2LS, UK.** (8008)A

ANNOUNCEMENT

Recruitment of Executive Director

The International Union Against Cancer, a non-governmental organization, wishes to recruit an Executive Director for its secretariat in Geneva.

Applicants should be knowledgeable in medical affairs, especially in the field of cancer and preferably in the international field. Experience in administration and financial management will be an advantage. A pleasant and diplomatic character trait would be helpful in our fund raising activities.

Languages: fluent English and a good working knowledge of French are expected.

Date of engagement will be decided by mutual agreement.

Applications with a curriculum vitae (photograph), photocopies of diplomas and degrees, and the names of two referees should be sent to:

Dr. Max. M. Burger
International Union
Against Cancer
3, rue du Conseil-Général
CH-1205 Geneva
(W5713)A

DYSON PERRINS LABORATORY

Postdoctoral Positions in Protein Studies

Applications are invited for two postdoctoral positions supported by an SERC grant to study protein protease inhibitors. One person will be involved in the synthesis, expression and site directed mutagenesis of genes in yeast or *E. coli*, and the other will be concerned with the isolation of the heterologous proteins and study of their inhibitory properties. Applicants must be familiar with either recombinant DNA techniques or have experience in protein isolation and enzyme kinetics.

The positions are available from 1 April 1989 for up to three years, but persons who would be available by 1 October 1989 including those in their final Ph.D. year are invited to apply. The salary will be on the Research Staff Grade 1A scale.

Applicants should send their *curriculum vitae*, and ask 2 academic referees to write on their behalf to Dr. G. Lowe FRS, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY (8009)A

POSTDOCTORAL POSITION

available spring 1989 to study in brain slices, serotonergic responses mediated by receptors postsynaptic to raphe neurons with special reference to hallucinogenic drugs. Experience with electrophysiological techniques required. Send curriculum vitae and names and addresses of three references to: **Dr. Joseph Goldfarb, Department of Pharmacology, Box 1215, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574.**

An Equal Opportunity Employer. (NW3054)A

CAMBRIDGE UNIVERSITY
VISUAL SCIENCE

Applications are invited for a two-year post-doctoral position for interdisciplinary research on the polymorphism of colour vision in primates and man. Postgraduate experience of electrophysiological recording would be preferred, but pre-doctoral candidates and candidates with training in other areas of visual science will be considered. The research is MRC-funded and is in collaboration with Dr. J. Bowmaker, Queen Mary College, University of London. Contact **Dr. J. Mollon, Department of Experimental Psychology, Downing St., Cambridge, CB2 3EB.** (8011)A

DEPARTMENT OF HEALTH

Chemical Toxicologists

London

You will be working in a combined medical and scientific division which is responsible for providing advice to the public and Government departments on potential hazards to health from environmental radiation and from chemical substances in food, drinking water, consumer products and the environment.

One post offers an opportunity to work on the safety evaluation of food additives and contaminants, currently an area of considerable public interest. The job involves assessing a wide variety of toxicological studies, giving a broad basis of all round experience in the science.

The other post offers an opportunity to work on the chemical contamination of drinking water and the environment. The work involves evaluation of toxicological and epidemiological data in an area where there is rapidly growing public, political and scientific interest.

Both posts involve work in the general area of regulatory toxicology, and post holders will make a wide range of contacts and will be closely involved with the scientific basis of regulatory decision making. The posts therefore provide an appropriate step in a wide range of careers.

You should have a 1st or 2nd class honours degree in a relevant science discipline and must have at least 2 years' experience in toxicology and/or environmental sciences.

Salary is in the range £12,255–£15,745 with further increments, depending on performance, up to £18,125 (including an Inner London Weighting of £1750).

For further details and an application form (to be returned by 20 January 1989) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours).

Please quote Ref: SB/21/KE.

The Civil Service is an equal opportunity employer

Scientific
CIVIL SERVICE
(7998)A

EVOLUTIONARY BIOLOGIST

EMORY UNIVERSITY, Department of Anthropology and Department of Biology, seek two joint positions in evolutionary biology. Candidate's research may be in any aspect of evolutionary biology. Ability to interact effectively with faculty in both Anthropology and Biology programs essential. Both positions are tenure track at the assistant professor level; for exceptional candidates, appointment as associate or full professor possible. Successful candidates must have a strong research record and a commitment to quality teaching. Review of applications will begin January 16, 1989. Send *curriculum vitae* and names and references to:

Peter J. Brown and Gray Crouse, Search Committee Chairs, Department of Anthropology, Emory University, Atlanta, GA 30322.

Emory University is an affirmative action/equal opportunity employer.
(NW3045)A

THE UNIVERSITY OF UTAH
Postdoctoral Fellow/Research Associate in Neurobiology

A position for a Postdoctoral Fellow or Research Associate in Neurobiology is currently available. Supported by an NIH grant through 1993, the project is studying interactions between axons and their target neurons in the developing auditory nervous system (see *Curr. Top. Develop. Biol. 21*:309-340, 1987). The project involves experimental embryology tissue culture, recording from and labeling neurons in brain slices, pharmacophysiology, and morphometric analyses. Experience in electrophysiology or tissue culture of neurons is desirable. The position offers competitive salary and benefits. The University of Utah is an equal opportunity employer. Please send a CV and the names of three evaluators to: **Dr. T. N. Parks, Department of Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.** (NW3074)A

Department of Geology
University of Alberta

Applications and nominations are invited for a Professorship in the area of Experimental Igneous Petrology. The Department of Geology is a well-equipped research oriented department in the Faculty of Science. The successful candidate will be responsible for supervising a research facility which includes a uniaxial, split-sphere USSA apparatus ("Superpress") a piston cylinder apparatus, and internally heated, gas pressure vessel and cold seal bombs.

We are seeking a candidate with excellent leadership qualities, an outstanding research record, and a dedication to teaching at the graduate and undergraduate level.

The position is now available with the appointment salary and rank commensurate with experience. Applications or nominations, including a detailed *curriculum vitae*, a brief statement of teaching and research interests, and the names of three referees, should be received by March 1, 1989 and addressed to:

Dr. N. W. Rutter, Chairman
Department of Geology
University of Alberta
Edmonton, Alberta, Canada
T6G 2E9

The University of Alberta is committed to the principle of equity in employment but, in accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. (NW3050)A

RMIT AN EQUAL OPPORTUNITY EMPLOYER

PRINCIPAL LECTURER

MEDICAL LABORATORY SCIENCE

AUSTRALIA

The Department of Applied Biology is a multi-disciplined department within the Faculty of Applied Science.

The appointee will be one of three (3) Principal Lecturers in the Department and be responsible to the Head of Department for co-ordinating the under-graduate and post-graduate degree courses in Medical Laboratory Science. In addition he/she will be involved in the general academic and administrative activities of the Department. It is expected that the appointee will teach and undertake research in his/her discipline and will be involved in collaborative industrial projects.

Applicants should possess an appropriate tertiary qualification to doctoral level. Teaching, medical diagnostic laboratory and research experience are essential. Some industrial and managerial experience is highly desirable.

Salary: $A49,704 p.a. **Ref:** 122/21/A.

Further information may be obtained from Dr R. Borland, Head of Department, on (61-3) 660 2612.

SENIOR LECTURER IN PATHOLOGY

The Department of Applied Biology is seeking a graduate in medical or veterinary science with appropriate post-graduate qualifications and clinical experience in pathology. Teaching and research experience would be desirable.

The successful applicant will have responsibility for teaching General Pathology and Special Pathology to second and third year undergraduates respectively in the Bachelor of Applied Science (Medical Laboratory Science) programme. In addition, the appointee will be expected to contribute to the coursework Master of Applied Science degrees in Applied Toxicology and in Clinical Laboratory Science.

Salary Range: $A40,937-$47,564 p.a. **Ref:** 122/25/A.

Further information may be obtained from Dr R. Borland on (61-3) 660 2612.

Phone Personnel Services on (61-3) 660 2337 for a Position Description. Written applications quoting relevant reference number should be addressed to The Senior Appointments Officer by 10 February, 1989. (W5707)A

ROYAL MELBOURNE INSTITUTE OF TECHNOLOGY LIMITED
GPO Box 2476V, Melbourne, Vic. 3001

SENIOR MOLECULAR BIOLOGIST

Lactic acid bacteria

A senior scientist with a proven record of innovative research in microbial genetics is offered a position on a project titled "The genetics of the genus Bifidobacterium". The succesful applicant will join a newly established group at the Danish Government Research Institute for Dairy Industry working on the genetics of lactic acid bacteria. Supervision of graduate (Ph.D.) work on projects associated with the appointee's own research is part of the job profile. In this respect the position is a guest professorship at **The Technical University of Denmark.**

The position is a two year research appointment administered by the government funded "Centre for Food & Process Biotechnology". Applications are requested as soon as possible and immediate appointment in the position is preferred.

The annual salary will be approx. US $ 30,000 — according to experience. In addition to the salary, a four-room modern flat will be offered.

Applications, including c.v., should be sent to:

Professor, Dr.techn. John Villadsen,
Director
Centre for Food & Process Biotechnology
Dept. of Biotechnology, Block 223
Technical University of Denmark
DK — 2800 Lyngby.
Tel: +45–2–88 40 66
Fax: +45–2–88 22 39

The Centre for Food & Process Biotechnology consists of 27 research projects and administers a grant equivalent to approximately US $ 8 mio.

The Danish Government Research Institute for Dairy Industry is at the town of Hillerød, 35 km north of the centre of Copenhagen.

(W5709)A

KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH

Kuwait Institute for Scientific Research (KISR) is a nonprofit organization with a staff of more than one thousand employees engaged in applied research in the fields of environmental and earth sciences; water resources; food resources; engineering; petroleum, petrochemicals and materials; and techno-economics.

KISR has a vacancy for a:

DATE PALM TISSUE CULTURIST

● Major Duties

— Developing plant tissue culture methodology for micropropagation research on date palms, with eventual inclusion of other plant species.
— Supervising and coordinating plant tissue culture laboratory activities and providing training in plant tissue culture methodology.
— Optimizing micropropagation systems for ultimate commercial application.
— Conducting intensive plant research including nutrition, metabolism, and environmental tolerance.
— Overseeing and guiding greenhouse acclimation and establishment of micropropagated plants.

● Qualifications

Applicants should have a Ph.D. in one of the basic plant sciences (Botany, Breeding, Physiology) with emphasis on tissue culture and/or advanced molecular genetics and its application in developing new cultivars. Strong background in plant physiology is an advantage. Five or more years of research experience in tissue culture with documented expertise in date palm tissue culture (somatic embryogenesis and/or organogenesis) as well as use of somaclonal variation in developing stress tolerant crop plants. Working experience overseas is an advantage.

KISR offers attractive tax free salaries commensurate with qualifications and experience and generous benefits that include gratuity, free furnished air-conditioned accommodation, school tuition fees for children, contribution to a savings plan, six weeks annual paid vacation, air tickets, free medical care and life insurance.

Interested applicants are requested to send their Curriculum Vitae with supporting information not later than one month from the date of this publication, to:

Director General, Kuwait Institute for Scientific Research
P.O. Box 24885 Safat, 13109 Safat — KUWAIT.

(W5711)A

The French National Center for Scientific Research (C.N.R.S.) is recruiting 609 researchers.

Applications to be received until 31 January 1989 at 6 pm [required level thesis].

Applications can be withdrawn before 31 January.

The best in every discipline will be shortlisted.

Enquiries about the different disciplines, positions and locations can be obtained from:

Centre national de la recherche scientifique
Direction du Personnel et des Affaires Sociales
Service des Carrières
Bureau des Concours Chercheurs
15, quai Anatole France
75700 Paris
FRANCE
Phone: [010.33] (1) 45.55.92.25
ext 2582–2565

(W5723)A

IRF
Institutet för rymdfysik
Swedish Institute of Space Physics

VISITING REMOTE SENSING SCIENTIST MICROWAVE SCATTERING STUDIES

The Swedish Institute of Space Physics (formerly known as the Kiruna Geophysical Institute) invites applications for a post-doctoral research position. The position is a time-limited one and will be available during the leave of absence of the head of the Radio Remote Sensing group, initially for a duration of six to twelve months, starting July 1, 1989. An extension may become possible.

The successful applicant will be a member of our team for microwave scattering studies. This was established about three years ago as a part of the national effort in support of the ESA ERS programme. During the next few years, emphasis will be put on experimental scattering and cross section studies of the land and vegetation typical of Northern Scandinavia and the interpretation of data in terms of scattering models as well as operational SAR signatures. Frequent co-operation with other groups engaged in SAR and microwave remote sensing is anticipated. He/she is also expected to provide scientific guidance to the junior members of the group in his/her fields of expertise.

The Institute seeks applications from highly motivated and creative individuals with several years' experience in the microwave remote sensing field. A background in SAR techniques is desirable and experience with polarimetry methods would be an advantage. Interested candidates should send an application with curriculum vitae, list of publications and names of three professional references to **Professor Bengt Hultqvist, Swedish Institute of Space Physics, P.O. Box 812, S-981 28 KIRUNA, Sweden,** before April 1, 1989. (Phone + 46-980-12240, Telex 8754 IRF S, Telefax +46-980-15465). The salary level depends upon experience and qualifications, starting from around SEK 16,000/month (corresponding to US $ 32,000/year).

The Swedish Institute of Space Physics is a separate Government agency and an equal opportunity employer.

(W5714)A

Public Service Commission of Canada | Commission de la Fonction publique du Canada

Research Scientist

Canadian Forestry Service
Northern Forestry Centre
Edmonton, Alberta

We require a molecular geneticist to initiate, develop and conduct research in molecular genetics using recombinent DNA technology, gene fusion and gene transfer in both pro- and eucaryotic organisms. The work involves analysis, interpretation and reporting of investigations at the scientific and technical report level and interaction and communication with other scientists from a variety of organisations.

You require a Ph.D. in Molecular Genetics or Biochemistry with several years of related experience preferably in Eucaryotic Systems and demonstrated ability to plan and conduct research, prepare scientific reports and communicate effectively.

Knowledge of English is essential.

We offer a salary commensurate with your qualifications and experience.

The Northern Forestry Centre is the western and northern regional office of the Canadian Forestry Service. Work will actually be conducted at the Plant Biotechnology Centre, University of Alberta under the guidance of Dr. A.A. Szalay.

For further information, please contact either Dr. S. Malhotra, Northern Forestry Centre, 5320-122 Street, Edmonton, Alberta, Canada, T6H 3S5; Phone (403) 435-7201, or Dr. A.A. Szalay, Department of Cell Biology, Medical Sciences Building, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7; Phone (403) 450-5327.

Forward your résumé and/or application form and the name of three references to:
John Taylor
Agriculture Canada
Personnel Branch
Room 227, Sir John Carling Bldg.
Ottawa, Ontario, K1A 0C5
Please Quote: 88-RES-62

Closing date: January 27, 1989
Personal information you provide is protected under the Privacy Act. It will be held in Personal Information Bank DOA/P-SE-902, Personnel Selection files.

(W5703)A

The Public Service of Canada is an equal opportunity employer

Canada

Tufts University, School of Medicine

POST DOCTORAL POSITION

Post doctoral position available immediately for Molecular Biologist/Biochemist to study transcription and translation factors regulating ferritin synthesis in normal and diseased states. Available for up to 3 years. Please send CV and letters of recommendation to

Dr. Jim Drysdale,
Dept. of Biochemistry,
TUFTS UNIVERSITY,
School of Medicine,
136 Harrison Ave.,
Boston, MA 02111.

(NW3078)A

Faculty Position in Soil Science

The School of Forestry & Environmental Studies at Yale University invites applications for a faculty appointment, at the Assistant Professor level, in soil science. Applicants should have a doctorate in some aspect of soil science. Teaching responsibilities will include a comprehensive, masters level course in soil science for natural resource management, and one or more advanced graduate courses. Research interest in forest soils or soils of other natural communities is especially appropriate. Specific focus, however, could be in the physical, chemical, or biological aspects of soil science.

To apply, send a letter stating teaching and research goals, a curriculum vitae and transcript, the names addresses, and phone numbers of three references, and examples of recent work to: **Prof. William H. Smith, Soils Search Chair, Yale University, School of Forestry & Environmental Studies, 370 Prospect Street, New Haven, Connecticut 06511, phone: (203) 432-5149.** A complete application packet should arrive no later than March 1, 1989 and is for an initial term of three years.

Yale University is an Equal Opportunity/ Affirmative Action Employer

(NW3072)A

CYTOGENETICIST, MASSACHUSETTS GENERAL HOSPITAL

We invite applications for a staff position in cytogenetics. Applicants should have a Ph.D. or M.D. degree, experience in cytogenetics (preferably with certification in clinical cytogenetics), a strong background in molecular biology, and research accomplishments. The person appointed will be Associate Director of the Cytogenetics Laboratory at the MGH and Assistant or Associate Professor of Pathology at Harvard Medical School. Send curriculum vitae and the names of three persons from whom recommendations can be solicited to

Dr. Robert T. McCluskey,
Department of Pathology,
Massachusetts General Hospital,
Boston,
MA 02114.

The Massachusetts General Hospital and Harvard Medical School are Affirmative Action/Equal Opportunity Employers. (NW3042)A

RESEARCH ASSOCIATE

To conduct research on the broad and specific respiratory controls of gene expression in *Escherichia coli*. The work will require knowledge and experience in enzymology, membrane biochemistry, intermediate metabolism, bacterial genetics, and various techniques of in vitro and in vivo recombinant DNA technology.

At least 3 years of postdoctoral experience with high quality of published work required.

Annual salary $32,000 plus benefits. Submit cv and names of three references to the **Division of Employment Security, Hurley Bldg., Government Center, Boston MA 02114,** and refer to job reference number 9262. (NW3064)A

ASSISTANT PROFESSOR MOLECULAR NEUROBIOLOGY UNIVERSITY OF FLORIDA COLLEGE OF MEDICINE

A tenure tract assistant professor position is available in the Department of Neuroscience starting on or before July 1, 1989. The University has a large and growing state-funded Interdisciplinary Center for Biotechnology Research and, together with the Department of Neuroscience, has a strong commitment to expand molecular biology research. The successful candidate will be expected to establish an independent research program and participate in graduate/medical teaching. Send a summary of research interests, curriculum vitae and three letters of recommendation to: **Dr. Gerry Shaw, Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610-0244,** before March 1, 1989.

The University of Florida is an equal opportunity/affirmative action employer. (NW3076)A

DIRECTOR, WISCONSIN REGIONAL PRIMATE CENTER

The University of Wisconsin-Madison announces a search for Director of the Wisconsin Regional Primate Center. The Director will be expected to lead development of strong research programs involving University Faculty and Staff and outstanding visiting researchers in important biomedical areas in which the non-human primate offers unique opportunities for endocrinological, reproductive and behavioural research relevant to the health of man and other primates. The director will also hold a tenured professorship in an appropriate academic department and will have the opportunity to continue his/her research and graduate student/postdoctoral training. Applicants should supply a full curriculum vitae, a statement of research interests and names of three references no later than February 1, 1989.

Address correspondence to Primate Director Search Committee, 333 Bascom Hall, University of Wisconsin-Madison, Madison, Wisconsin 53706.

The University is an equal opportunity/affirmative action employer. (NW3086)A

UNIVERSITY OF SURREY
DEPARTMENT OF MICROBIOLOGY
RESEARCH IN MOLECULAR VIROLOGY
RE-ADVERTISEMENT

A research post funded by Action Research for the Crippled Child is available for up to three years to study the molecular biology of rubella virus.

We are looking for a post-doctoral scientist, preferably with experience and a continuing interest in virology, although a suitably qualified graduate will be considered.

The successful applicant will join Dr P G Sanders' group at Surrey in a collaborative study with Dr J Best and colleagues at St Thomas' Hospital, London.

The post is available immediately and the starting salary will be up to £11,680 pa. Starting salary will depend on age, experience and qualifications and superannuation will be under USS conditions. For further details contact Dr P G Sanders on (0483) 571281 ext 2665.

Applications in the form of a Curriculum Vitae (3 copies) including the names and addresses of two ref erees, should be sent to the **Personnel Officer (JLG), University of Surrey, Guildford, Surrey GU2 5XH,** by 27th January 1989 quoting the reference number:: 797N.

Previous applicants for post number 755 are still under consideration. (8015)A

Faculty Position in Geographic Information Systems

The School of Forestry & Environmental Studies at Yale University invites applications for a faculty appointment, at the Assistant Professor level, in geographic information systems. Applicants should have completed their doctorate by the date of appointment in a relevant field such as Forestry, Geography, Geology, Remote Sensing, Landscape Architecture, Ecology, or Computer Science. Teaching responsibilities will include a comprehensive, masters-level course in GIS for natural resource management, and one or more advanced graduate courses. Research interests in applications or development of GIS for interdisciplinary natural resource management are preferred. Opportunities for collaborative work in parallel computation exist with the Department of Computer Science. To apply, send a letter stating teaching and research goals, a curriculum vitae and transcript, the names, addresses, and phone numbers of three references, and at least one example of recent work (e.g., reprints, manuscripts, or research proposals) to: **Prof. Clark S. Binkley, GIS Search Chair, Yale University, School of Forestry & Environmental Studies, 205 Prospect Street, New Haven, Connecticut 06511, phone (203) 432-5136.** A complete application packet should arrive no later than March 1, 1989. The position is available July 1, 1989 and is for an initial term of three years.

Yale University is an Equal Opportunity/Affirmative Action Employer (NW3083)A

UNIVERSITY OF MICHIGAN
Department of Geological Sciences

Postdoctoral Research Assistantship in Radiogenic Isotope Geochemistry

Applications are invited for a 3-year position (subject to renewal) funded by the University of Michigan. Candidates should have experience in Nd, Sr or Pb isotope geochemistry and thermal ionisation mass spectrometry.

Instrument Analyst in Radiogenic Isotope Geochemistry

Applications are invited for a technical position in $^{40}Ar-^{39}Ar$ geochronology. The position is for two years subject to renewal. Candidates should have a Masters degree in physics, chemistry or geology and experience in mass spectrometry and vacuum techniques.

Applicants for either post should send a resumé and names and addresses of at least three references by February 15, 1989, to **Dr. Alex Halliday, Department of Geological Sciences, University of Michigan, 1006 C.C. Little Building, Ann Arbor, MI 48109-1063.**

The University of Michigan is an Equal Opportunity/Affirmative Action Employer. (NW3063)A

RESEARCH ASSISTANT

SOMATIC CELL GENETICS

CAMBRIDGE UNIVERSITY,
UK/ELEANOR ROOSEVELT INSTITUTE, DENVER, USA

Research Assistant required for joint project between a Cancer Research Campaign funded group in the UK and the Eleanor Roosevelt Institute, Denver, USA. The purpose is to develop somatic cell hybrid resources for mapping the gene for multiple endocrine neoplasia on human chromosome 10. The successful applicant will be seconded 6-12 months to the laboratory of Dr. Carol Jones in Denver before returning to the UK to the CRC-funded group which is expected to move to new laboratories in Cambridge in Spring 1989. Substantial previous experience in tissue culture is essential. The post is CRC funded; salary and conditions on Cambridge University scales £7,515-£12,760 for Research Assistant or £11,680-£14,500 for Research Associate. Duration of post not less than 3 years according to age and experience of applicant. Starting date early 1989 negotiable. Informal enquiries to Dr. Bruce Ponder (01-643 8901 ext 4693) or Section of Human Cancer Genetics, Institute of Cancer Research, Sutton, Surrey SM2 5NG.

Applications in duplicate to the **Personnel Officer, Institute of Cancer Research, 17a Onslow Gardens, London SW7 3AL** quoting ref. no. 12.88.T.NS.59. (7983)A

TRUDEAU INSTITUTE, INC.

POSTDOCTORAL ASSOCIATE TO STUDY T CELL-MEDIATED IMMUNITY

A vacancy exists for a postdoctoral fellow interested in studying immunity to tumor and histocompatibility antigens. Current studies deal with various aspects of T cell-mediated rejection of tumors and allografts. The opportunity to study T cell-mediated immunity to bacterial infection also exists. Applicants should have basic training in immunobiology. Starting salary will be commensurate with experience and includes liberal fringe benefits. Subsidized institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake and near Lake Placid, N.Y.

Send curriculum vitae and names of three references to:
Dr. Robert J. North, Director Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983. (NW3080)A

MOLECULAR GENETICIST

(Gene Mapping)

DIVISION OF TROPICAL ANIMAL PRODUCTION

TROPICAL CATTLE RESEARCH CENTRE
ROCKHAMPTON, QUEENSLAND
AUSTRALIA

A$31003 - A$38045

PROJECT: The project will establish a primary bovine genetic map in order to develop gene probes for cattle. The two phases involve firstly the establishment of DNA banks from selected reference families. Secondly RFLP linkage mapping will be carried out within the reference families. It is envisaged that the isolation of bovine specific hypervariable sequences will be required. Additional linkage studies using reference families will be conducted by collaborating groups throughout the world.

DUTIES: Specific duties will include selection and isolation of genes/markers to be mapped, RFLP studies within the reference families and liaison with collaborating groups. Interaction with other molecular genetics projects is envisaged.

QUALIFICATIONS: Applicants should have a Ph.D or equivalent qualification in molecular biology/genetics or related fields. Experience in RFLP linkage mapping, cloning and comparative gene mapping is desirable.

CONDITIONS: The initial appointment will be for a period of three years with Australian Government Superannuation benefits available. Assistance with relocation will be offered.

APPLICATIONS: Stating relevant personal particulars including details of qualifications and experience, the names of at least two professional referees and quoting reference number (RDT02) should be directed to:

The Chief,
CSIRO Division of Tropical Animal Production,
Box 5545,
Rockhampton Mail Centre,
Queensland 4702
AUSTRALIA
Fax: 6 17 079 36 1288

Within four (4) weeks of the date of this publication.

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W5717)A

MOLECULAR GENETICS
Three positions

We are looking for three individuals to staff a new molecular genetics and *in situ* hybridization unit at the Whitney Laboratory. One position will be at the senior postdoctoral level and will be filled by someone with broad experience in various aspects of molecular genetics. This individual will be responsible for overseeing the unit and providing technical advice to other investigators in the Laboratory. The two other positions are at the junior postdoctoral level. One will have experience in the techniques of molecular genetics and a desire to learn electrophysiology; the other will have experience in electrophysiology (preferably intracellular recording and whole-cell and single-channel voltage clamp) and a desire to learn molecular genetics.

The Whitney Laboratory is the Institute for Marine Biomedical Research and Biotechnology of the University of Florida, and an Equal Opportunity Employer. Contact: **Dr. Peter A.V. Anderson, The Whitney Laboratory, 9505 Ocean Shore Blvd., St. Augustine, FL 32086.** (NW3047)A

The Polytechnic operates a range of degree and post graduate level courses, has a vigorous and successful record of consultancy and research and with the advent of Corporate Status the Polytechnic is entering a new and challenging phase of its development. To maintain and extend the programme of work, applicants are invited for the following post:

SCHOOL OF PHARMACEUTICAL & CHEMICAL SCIENCES

Head of School

Salary – HOD VI – £24,084 - £26,538

Applicants for the post should possess high academic credibility and also have the ability to manage the School as a budget centre, be able to lead in academic development and entrepreneurial initiatives. The person appointed would be eligible for consideration for appointment to the Professoriate.

The School of Pharmaceutical and Chemical Sciences is one of 3 Schools in the Faculty of Science and has an establishment of 30 academic staff. The School embraces the disciplines of chemistry and pharmacy (except for pharmacology).

Informal contact: Professor J. R. Brown, Dean of Faculty (091) 5152504.

Application forms and further particulars can be obtained from the Personnel Officer, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland, SR2 7EE or telephone (091) 5152429.

Closing date: 13.2.89. (8018)A

SUNDERLAND POLYTECHNIC

MICROBIAL ECOLOGY ASSISTANT PROFESSOR HARVARD UNIVERSITY

The Department of Organismic and Evolutionary Biology, Harvard University, invites applications for a faculty position in the field of microbial ecology, with special emphasis on symbiotic interactions between microorganisms and higher organisms. Candidates should have demonstrated excellence in research and a strong commitment to teaching at the undergraduate and graduate levels, including participation in courses in microbial diversity and ecology. Applications including a curriculum vitae, statement of research interests, reprints, and three letters of reference should be sent by March 1, 1989, to:

Dr. Andrew H. Knoll
Chairman Search Committee in Microbial Ecology
Department of Organismic and Evolutionary Biology
Harvard University
26 Oxford Street
Cambridge, Ma. 02138

Harvard University is an Affirmative Action/Equal Opportunity Employer. (NW3082)A

Friedrich Miescher-Institut Basel, Switzerland.

POSTDOCTORAL FELLOW

A postdoctoral position is open for a biochemist to join the group of Dr. Josef Jiricny. The project entails the isolation and characterization of human DNA repair proteins. The successful candidate should be familiar with the techniques of protein purification. Previous postdoctoral experience is desirable but not essential.

The Institute offers excellent research facilities. The appointment would be initially for one year, with extension foreseen for a further two years. Applications enclosing a curriculum vitae and a list of publications, as well as the names and addresses of two academic referees, should be sent to:

Dr. Josef Jiricny,
Friedrich Miescher-Institut,
PO Box 2543, CH-4002 Basel,
Switzerland.
(Tel: 061-6976661
or 6975046).

(W5645)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH
DIVISION OF BIOTECHNOLOGY

MICROBIOLOGIST

A graduate technician is required to join the Thermophile Research Section. The successful applicant will be involved in genetic and physiological studies of a wide range of thermophilic microorganisms.

This appointment will be at Basic Grade Microbiologist or Medical Physics Technician grade IV, depending upon qualifications and experience.

Salary range: £6,763-£10,496 (pay award pending).

NHS terms and conditions.

Candidates wishing to informally discuss the post should contact Dr R. J. Sharp, tel: Idmiston (0980) 610391, ext. 443.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Tel: (0980) 610391, to whom completed forms should be returned by 23rd January 1989 quoting Post No. 0809. (7997)A

THE UNIVERSITY OF SYDNEY

Australia

Applications and expressions of interest are invited for the following Chairs in the Faculty of Veterinary Science which will become vacant in January 1990.

CHAIR OF VETERINARY PATHOLOGY

Reference no. 48/19

The Department is responsible for teaching, research and diagnostic services in the field of Clinical Pathology, Microbiology and Parasitology. Veterinary qualifications or experience in teaching pathology to Veterinary Science students would be desirable.

CHAIR OF VETERINARY PHYSIOLOGY

Reference no. 48/03

Veterinary qualifications or experience in teaching physiology to Veterinary Science students would be desirable.

Intending applicants should obtain detailed statements of information concerning the Chairs from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Salary will be at the rate of A$63,397 per annum.

The University reserves the right not to proceed with any appointment for financial or other reasons.

Applications, in duplicate, quoting reference no., including curriculum vitae, list of publications and the names, addresses and FAX numbers of three referees, to the Registrar, Staff Office, University of Sydney, NSW 2006, Australia (FAX +612 692 4316).

Applications close: 8 March 1989.

Equal employment opportunity is University policy. (W5712)A

POSTDOCTORAL FELLOW — MOLECULAR BIOLOGY OF LUNG DEVELOPMENT. Position available 1 July 1989 in NIH-supported laboratory program to study mechanisms of hormone effects on gene expression during development of lung cells. Qualifications: Ph.D. with background in molecular biology. Please contact: **Dr. Donald L. Shapiro, Department of Pediatrics (Box 651), University of Rochester School of Medicine, 601 Elmwood Avenue, Rochester, NY 14642. Telephone: 716 275-5884.** *An Equal Opportunity Employer M/F.* (NW3073)A

ACADEMIC PHYSIOLOGIST

The Department of Anesthesiology of Loyola University Stritch School of Medicine, Maywood, Illinois is recruiting a physiologist for a faculty position at the Assistant Professor level to work in a stimulating environment that includes excellent research laboratory. The candidates for this position are expected to have a strong commitment to innovative research in cardiovascular and respiratory physiology and ability to run large animal experiments in collaboration with the departments physician faculty and resident trainees. The salary is competitive and Loyola University is an Equal Opportunity Employer.

Please submit curriculum vitae, reprints, a statement of research interests, and arrange to have three letters of recommendations sent directly to:

Tadikonda L.K. Rao, M.D
Department of Anesthesiology
Loyola University
2160 S. First Avenue
Maywood, Illinois 60153 (NW2895)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
DEPARTMENT OF CLINICAL PHARMACOLOGY

SENIOR RESEARCH OFFICER (postdoctoral): pharmacology/physiology

Applications are invited from postdoctoral scientists for an MRC funded position in an interdisciplinary research group studying the role of cell infiltration and mediator release in the lungs in asthma. The post provides excellent opportunities for working at all stages of the pharmacology and physiology of asthma, from basic biochemical mechanisms through animal studies to man. Applicants should have a Ph.D. in pharmacology, physiology or a related discipline.

The post is funded on an MRC programme grant until May 1991.

Salary on Range 1A.

Application forms and further particulars are available from the **Personnel Officer, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN (tel: 01-740 3204)** quoting ref: **AEB2.**

Closing Date: 19.1.89. (7978)A

Emory University
Woodruff Chair in Molecular Genetics

Emory University announces the creation of an endowed chair; the Woodruff Professorship in Molecular Genetics. An interdepartmental program in Genetics exists in the Division of Biological Sciences and currently consists of over 25 faculty. Research interests represent most areas of modern molecular genetics encompassing a wide variety of systems ranging from the human to viral organisms and there is considerable collaborative interaction among the genetics faculty. The chair will be an independent unit reporting directly to the Dean of the School of Medicine and will include additional new faculty positions and an initial 5,000 square feet of newly constructed laboratory space as well as competitive seed funds and salary. Investigators with active research programs capable of providing campus-wide leadership in research and training in this discipline, including established investigators as well as those with unusual promise and a developing international reputation, are encouraged to apply.

Submit applications or nominations by February 15, 1989 to: **Woodruff Molecular Genetics Search Committee, Emory University School of Medicine, 1440 Clifton Road, NE, Atlanta, GA 30322.**

An Affirmative Action/Equal Opportunity Employer (NW3077)A

UNIVERSITY of GUELPH

CHAIR — DEPARTMENT OF ZOOLOGY
COLLEGE OF BIOLOGICAL SCIENCE

Nominations and applications are invited for the Chair of Zoology in the College of Biological Science. The candidate should be an outstanding scholar with a strong commitment to excellence in graduate and undergraduate education. The Department of Zoology has 30 faculty members with diverse research interests, particularly in ecological and physiological aspects of fisheries, marine, and wildlife biology. Approximately 60 graduate students are enrolled for the MSc/PhD degrees and 300 students are specializing in the four departmental undergraduate programs.

The College of Biological Science is currently reorganizing its curriculum. It requires an academic with strong leadership skills as the Chair of the Department of Zoology to meld the considerable strengths within the department, to recruit new faculty, and to provide direction for the 1990's.

Candidates should submit a curriculum vitae, a list of publications, and the names of at least 3 referees to:

Dr. Bruce H. Sells
Dean
College of Biological Science
University of Guelph
Guelph, Ontario
Canada N1G 2W1

Deadline for receipt of applications is February 28, 1989. Subject to final budgetary approval.

In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents.

The University is committed to Employment Equity.

(NW3021)A

UNIVERSITY OF SURREY
DEPARTMENT OF BIOCHEMISTRY

Postdoctoral Research Fellow (Project Leader) Nursing Practice Unit

Applications are invited for the post of Project Leader in the above Unit.

The NPRU is engaged on a number of fundamental research projects involving a range of biological and social sciences. The successful applicant will join an established programme of research studying aspects of hospital acquired infection. The person appointed will be responsible for the completion of the current project investigating the aetiology, incidence and prevention of catheter associated bacteriuria and for initiation of further research related to the use of invasive medical devices.

Applicants from a wide range of academic disciplines will be considered but possession of a PhD is essential. The post will be available for 18 months in the first instance. Salary will be up to £13870 per annum on the Research and Analogous Scale 1A, according to age, qualifications and experience.

Applications in the form of a curriculum vitae (3 copies) including names and address of two referees should be sent to the **Personnel Office (JLG), University of Surrey, Guildford GU2 5XH,** quoting reference 750aN by 20 January 1989. Further details may be obtained from Professor R Crow or Dr A Mulhall, 0483 571281 Ext 2549 or 2566. (8016)A

AFRC INSTITUTE OF FOOD RESEARCH, READING LABORATORY, SHINFIELD READING

Opportunities in protein structure, design and engineering

Two positions are available within our active protein engineering group. One at postdoctoral level, is funded by the E.C. and is in collaboration with the University of Groningen. The other, funded by the AFRC, affords the opportunity to study for a PhD. and is in collaboration with Birkbeck College, London.

(1) Postdoctoral position for the rational modification of the specificity of phospholipase A2

Phospholipase A2 is an excellent model system for investigating protein-phospholipid interactions. Inhibitors of phospholipase are pharmaceutically important and modified enzymes have great industrial potential. We are now initiating molecular simulation and free energy calculations to complement our existing protein engineering effort. This project is part of a three-way collaboration involving the Universities of Utrecht and Groningen and Reading Laboratory.
The appointment is for two years in the first instance, available now, and is funded by the E.C. Biotechnology Action Programme.
Qualifications required: PhD. in molecular simulation, protein structure, biomolecular computing or related area.
Salary on scale: £12,445–£17,032
Please quote reference R35/88

(2) Postgraduate position in protein structure and design

The aim of this project is to develop methods for adequately accounting for the solvation of charged and polar groups by solvent and protein. Such considerations are vital for accurate protein design. There will be ample opportunity to explore other aspects of protein structure including the design of mutants and X-ray analysis of cyrstallized mutant protein. The successful candidate will be able to register for a PhD. at Birbeck College, London.
This three year appointment is funded by the AFRC and available now.
Qualifications required: First or Upper second class honours degree in chemistry, physics, biological sciences or computation.
Salary on scale: £10,026–£13,460
Please quote reference R18/88

Excellent computer graphics facilities are available in Reading Laboratory and there is good access to ample computational power.

Closing date: 20th January 1989

The AFRC is an equal opportunities employer. (8020)A

PHARMACOLOGY — MOLECULAR BIOLOGY

Applications are invited for a tenure-track faculty position in the Department of Pharmacology of the University of Alabama at Birmingham (UAB). Comprising the Medical Center, University College and the Graduate School, UAB is a dynamic institution which is ranked as one of the top twenty comprehensive health and research centers in the U.S. The Pharmacology Department has a young well-funded faculty, is well-equipped and offers an excellent environment for research including competitive salaries, space and start-up funds. Applicants should have a relevant Ph.D. or M.D. degree, postdoctoral or faculty experience and be committed to a productive, independent research program and excellence in teaching. Rank is open but Assistant Professor is the likely level of appointment. We are seeking outstanding individuals whose interests and background are in the application of modern techniques of molecular and cellular biology and biochemistry to important problems in pharmacology. Send curriculum vitae, a brief statement of research interests and three letters of reference to: **Dr. Elias Meezan, Chairman, Department of Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294.** *UAB is an Equal Opportunity/Affirmative Action Employer.* (NW3084)A

THE UNIVERSITY OF NEW SOUTH WALES
Sydney, Australia

PROFESSOR OF CLINICAL PHARMACOLOGY AND DIRECTOR, DEPARTMENT OF CLINICAL PHARMACOLOGY AND TOXICOLOGY AT ST. VINCENT'S HOSPITAL

Applications are invited for appointment to the above position. Applicants should possess medical qualifications registerable in New South Wales and have appropriate teaching, research and clinical experience.

The Chair is in the School of Physiology and Pharmacology. The successful applicant will have similar standing in the School of Medicine. The Directorship of the Department of Clinical Pharmacology and Toxicology at St. Vincent's also involves responsibility for the New South Wales State Reference Laboratory for drug analysis and clinical responsibilities within that teaching hospital and for consultations in toxicology referred from other hospitals.

The Professor will participate in the undergraduate teaching programs in pharmacology clinical pharmacology, toxicology, and therapeutics on campus and at St. Vincent's Hospital and will also be involved in the development of these programs in the new Six Year medical curriculum. Responsibility for the coordination of teaching programs in these disciplines at the other teaching hospitals of the University of New South Wales is also involved. The appointee will be expected to engage in and promote research and advanced studies in the field of the Chair. Further information may be obtained from Professor W. E. Glover, Dean, Faculty of Medicine, or from Professor Ian McCloskey, Head, School of Physiology and Pharmacology.

The salary will be A$63,397 per annum and a clinical loading of A$11,176 per annum is also payable. There is an additional 'on call' allowance of A$13,003 per annum payable by the Hospital. There is also a 'clinical duties (Public Hospitals)' allowance payable by the Hospital of between A$9,390 and A$17,736 per annum depending on the level of administrative and service responsibility in the Hospital. Limited rights of private practice are also available. The University reserves the right to fill any Chair by invitation.

Details of the position, together with the conditions of appointment and application procedure are available from the Senior Appointments Unit, The University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033 Australia.

Applications close: 6 February 1989.

Equality of employment opportunity is University policy.

(W5702)A

UNIVERSITY OF HONG KONG
Department of Physiology

POSTDOCTORAL FELLOW IN PATCH-CLAMP STUDIES

Applications are invited, preferably from those with experience of patch-clamp technique, for a WHO-supported project on the role of ion channels in fluid secretion in the epididymis.

Appointment will commence in Spring 1989 and is for one year in the first instance with possible extension for a further two years. Annual salary is about US$20,000+, with medical benefits provided. A return airfare will also be provided if required. At current rates, income tax will not exceed 15.5 per cent of gross income.

Send curriculum vitae, statement of research interests, and the names of three referees to: Dr P Y D Wong, Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong. Tel: (852) 5-8199202, Telex: 71919 CEREB HX, Fax: (852) 5-479907. Closing date: January 31, 1989. (W5701)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PATHOLOGY

PREDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post available from 1 March 1989 for a fixed period of 36 months for work on RAS Mutations in Colorectal Cancer, involving the use of molecular biological techniques and the Polymerase Chain Reaction.

A good honours degree in Biochemistry or a related subject and experience in molecular biology would be an advantage.

Salary on R/A IB scale (£8,675-£11,680) according to qualifications and relevant experience.

Informal enquiries may be made to Dr Philip Quirke or Mrs J Fearnley (tel (0532) 333399 or 333387).

Application forms and further particulars may be obtained from the **Registrar, The University, Leeds LS2 9JT**, quoting reference no. **102/36**. Closing date for applications **31 January 1989**. (8012)A

University of Nebraska Lincoln

FACULTY POSITION IN INFECTIOUS DISEASE RESEARCH

Twelve month tenure-track position. Should be interested in studying infectious disease pathogenesis, microbial virulence factors or host defense mechanisms. Training in bacteriology, immunology, molecular biology, pathology or virology is appropriate. Will be expected to develop a competitive research program and participate in training graduate students. Start-up funds, technical support, and excellent laboratory and animal isolation facilities (BL2 & BL3) are available. Requires PhD in an appropriate discipline, or a DVM with MS or PhD. Rank and salary will be commensurate with record of accomplishments.

Send curriculum vita, research interests/career goals, and three letters of reference by February 15 to: **Dr. John A. Schmitz, Head, Department of Veterinary Science, University of Nebraska-Lincoln, Lincoln, Nebraska 68588–0905. (402) 472–2952.**

Affirmative Action/Equal Opportunity Employer (NW3058)A

ASSISTANT PROFESSOR

Theoretical Ecologist or Theoretical Population Biologist. Applications are invited for a tenure track Assistant Professorship *beginning August, 1989.* Teaching responsibilities will include a theoretical ecology or population biology course and participation in undergraduate offerings. The University of Wisconsin-*Madison* is an Affirmative Action, Equal Opportunity Employer *and encourages women and minorities to apply.*

Please submit curriculum vitae, copies of publications, description of research and teaching interests, and have three letters of recommendation sent by 1 March 1989 to:

Chair, Population Biologist Search Committee
Department of Zoology
University of Wisconsin-Madison
Madison, Wisconsin 53706 USA
608-262-1087 (NW3065)A

University of York
Department of Biology

POSTDOCTORAL RESEARCH FELLOW

The above SERC-funded post is available immediately for a period of up to one year and nine months. The project involves molecular biological and biophysical characterization of the endomembrane ATPase H+ channel, particularly with respect to its evolutionary relationship to the F_0F_1 ATPase of energy-coupling membranes. The successful candidate will join an active group involved in research on various aspects of membrane transport.

Starting salary within the range £9,865 to £10,460 per annum, with USS.

Three copies of applications, with full curriculum vitae and naming two referees, should be sent by 27 January 1989 to Registrar's Department (Appointments), University of York, Heslington, York Y01 5DD. Please quote advertisement reference number 7/7148C.

Informal enquiries to Dr D. Sanders (tel. 0904-432825) or Dr P. Rea (tel. 05827-63133 ext. 2264). (7993)A

KING'S COLLEGE LONDON
BIOMEDICAL SCIENCES DIVISION

Lectureship in Pharmacology

Applications are invited for a post of Lecturer in Pharmacology in the Biomedical Sciences Division. Applicants should show clear evidence of research potential or, in the case of older applicants, of research achievement in a branch of Pharmacology.

Salary within Lecturer Scale Grade 'A' range £9,260 pa–£14,500 pa plus £1,650 London Allowance, or 'B' range £15,105 pa–£19,310 pa plus £1,650 London Allowance according to age, experience, etc.

Further particulars and application forms from **Mr G A Cuthbert, Personnel Department, King's College, Strand, London, WC2R 2LS.** Closing date 31st January, 1989. (8002)A

Physicist

Nuclear Structure Facility — Ref DL/84

Daresbury Laboratory operates a 20MV Tandem Van de Graaff Accelerator for heavy ion nuclear structure research. This international facility is used by research groups for the UK and abroad.

• The Job

A vacancy exists for a physicist in the NSF Injection Systems and Developments group. The group is responsible for the development and operation of a wide range of ion sources for the accelerator, including those for polarised and radioactive beams. Although primarily working on ion sources the appointee will also participate in the general development programme of the facility where a superconducting linear accelerator booster is being installed.

• The Person

The successful applicant will be a graduate with practical ability and capable of understanding and applying the various areas of physics involved. He/She will work in collaboration with more senior members of the group from whom direction will be given. Someone with experience of ion sources would be preferred. The successful candidate will show the ability to understand and learn new ideas quickly and would be expected to develop rapidly.

Technical information can be obtained from Dr K A Connell on 0925 603496.

Station Scientist

Synchrotron Radiation Research — Ref DL/85

There is a vacancy in the Synchrotron Radiation Research Division for a station scientist. The successful candidate would be attached to the X-ray Diffraction Project team at the SRS Daresbury.

• The Job

The successful applicant will be responsible for the design, development and operation of diffraction equipment on the X-ray beam lines of the SRS. In addition to assisting and collaborating with external users, the station scientist will co-ordinate the work of computer and technical support staff. Development projects include the optimization of station software, and the testing of specialised environmental stages such as furnaces, cryostats and pressure cells. The candidate will also be expected to take an active part in the planning of diffraction stations to be constructed on the new wiggler beam-line.

• The Person

Applicants should have a relevant degree or equivalent qualification. They should be familiar with X-ray diffraction techniques on powders or single crystals, and have a sound background in crystallography, plus a flair for the design of unconventional instrumentation.

Further information can be obtained from Dr P Pattison (Tel. 0925 603154) or Dr R J Cernik (Tel. 0925 603238).

• Pay

Appointments will be made at the Scientific Officer or Higher Scientific Officer grade depending on qualifications and experience. Salary is in the range £8574 to £13460 with further increments depending on performance.

The superannuation scheme is non-contributory.

For an application form please quote the relevant reference number and contact:
The Personnel Officer,
Daresbury Laboratory,
Warrington WA4 4AD.
Telephone (0925) 603467
(24 hour answering service)

CLOSING DATE: 20th January, 1989. (8023)A

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

University of Basel — Department of Zoology

Academic Position in Cell Biology

This is a non-renewable academic position available for up to 5 years. We require a cell biologist to join a group investigating an in vitro transdifferentiation and regeneration system based entirely on striated muscle cells of jelly fish. The candidate should have a Ph.D and a good background in biochemistry. His/her work will focus on the mechanisms which stabilise cells in the differentiated state. It will include biochemical analysis of extracellular matrix components and application of mAb and standard cell biological techniques to early transdifferentiating cells. Some teaching duties are included. The salary will be in the range Sfr. 50'000-70'000 per year and will depend on experience and qualifications. Habilitation will be possible. Starting date: 1.3.89 or by arrangement. Applications including a c.v. and the names of two referees should be sent to **Prof. Dr. V. Schmid, Zoologisches Institut, Rheinsprung 9, CH-4051 Basel, Switzerland** (W5716)A

REGULATORY SPECIALIST

GARST SEED COMPANY is a member of the Agricultural Products Group of Imperial Chemical Industries PLC (London), the world's fourth largest chemical manufacturer and a world leader in discovery, technology, and innovation. The dedication of a new Bioscience Laboratory in Slater, Iowa in October of this year marked the consolidation of Garst's traditional plant breeding program with the international bioscience capabilities of ICI.

The Regulatory Specialist will report to Garst's Executive Vice President and Director of Research, and lead ICI/Garst through the regulatory process -

- Obtaining government approval for all aspects of new seed products;
- Monitoring the development of relevant regulations in North America;
- Formulating an overall registration strategy.

Inherent tasks will include planning and assembling a suitable package, negotiating with Federal and State officials, dealing with the press and public, streamlining systems and procedures, and consulting with our Government Relations staff in Washington.

We are looking for demonstrated **competence** - in familiarity with the regulatory process at state and federal level and awareness of trends and developments; in being equally at home with scientists, production, and marketing people; in being an effective spokesperson in varied forums in the private and public sectors; in understanding the entrepreneurial flavor of our business; and in skillful negotiation and the legal process. Any combination of degrees and experience contributing thereto would be welcome.

Position is located in Slater, Iowa, and will entail extensive travel throughout US and Canada, and possibly Central and South America.

We offer a competitive salary commensurate with experience, comprehensive benefits, high professional standards and respect, and - most importantly - the career association with a world leader in meeting farmers needs for the coming decade. Our growth over the past decade has been nothing less than phenomenal, and we invite your candidacy. Principals only. Please submit resume in confidence to **Charles T. Brodigan, Associate Employment Manager, ICI Americas, Wilmington, DE 19897.**

ICI

World Problems,
World Solutions,
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GARST SEED COMPANY

(NW3067)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF PATHOLOGY

AIDS SECTION

POST-DOCTORAL POSITION FOR A PROTEIN BIOCHEMIST

A position is now available to study Human Immunodeficiency Virus proteins. The three year post is funded by the MRC AIDS Directed Programme and the salary range is £11,164-£14,591 per annum (under review) depending on age and qualifications. Previous experience with protein purification and immunochemistry is essential. Familiarity with virological and tissue culture techniques would be desirable but not essential.

National Health Service terms and conditions will apply.

Further details can be obtained from Dr G W Farrar.

Applications forms are available from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Telephone (0980) 610391, ext 240 to be returned by 20th January 1989 quoting Post No. 0322.

(8019)A

CONDENSED MATTER EXPERIMENTAL PHYSICISTS
COLUMBIA UNIVERSITY
DEPARTMENT OF PHYSICS

The Department of Physics of Columbia University is searching for outstanding condensed matter experimental physicists at the assistant professor level.

The successful candidates will be provided with startup funds to suit the requirements of their research programs. There is no restriction on the subfield that will be considered. Tenure positions will be available for some of the physicists in this program who develop truly outstanding research programs and who also demonstrate excellent teaching skills. We will also consider applications, at this time, from more senior experimental condensed matter physicists who presently have the qualifications for a tenure position.

Finally we will consider applications from small, established, highly successful groups of experimental physicists who might be interested in moving as a group to Columbia. These positions will remain open until they are filled. Persons or groups interested in being considered for these postions should send their curriculum vitae, publication list, and a statement of their research interests to:

Professor S. Nagamiya
Condensed Matter Search Committee Chairman
Department of Physics
Columbia University
Box 3 Pupin
New York, N.Y. 10027

They should also arrange to have, at least three letters of recommendation sent to the same address. Columbia University is an equal opportunity/affirmative action employer. Women and members of minority groups are encouraged to apply for these positions.

(NW2914)A

THE UNIVERSITY OF TENNESSEE
MEDICAL CENTER AT KNOXVILLE (UTMC/K)

2 FACULTY POSITIONS
(tenure-track)

Two (2) tenure-track faculty positions — **MEDICAL ONCOLOGIST/ CLINICAL INVESTIGATOR** and **MOLECULAR IMMUNOLOGIST/ PROTEIN BIOCHEMIST** — at the assistant/associate professor level for MD/PhD candidates available in the Department of Medicine's Human Immunology and Cancer Program. NIH- and ACS-supported clinical and basic science research with emphases on human light chain-associated renal and systemic diseases (multiple myeloma, amyloidosis, etc.) and B cell-related neoplasms (lymphoma, leukemia, etc.). The successful candidate will work in well-equipped, state-of-the-art basic and clinical research facilities including hybridoma/Mab, flow cytometry, protein sequence, and immunochemistry laboratories. These positions are immediately available and require a minimum of 3 to 5 years of postdoctoral experience and ability to conduct innovative research. For MD candidates, BC/BE and eligibility for state licensure required. Excellent compensation (including fringe benefits) and academic rank commensurate with education and experience. The Knoxville area offers the scientific resources of the Univ. of Tennessee and the Oak Ridge National Laboratory, recreational opportunities provided by the surrounding Great Smoky and Cumberland Mountains and extensive TVA lake system; and cultural amenities including a symphony and chamber orchestra, a major fine arts museum, opera, and theatre. Letter of application, CV, and names and addresses of 3 reference sources should be sent to: **Alan Solomon, M.D., Professor o Medicine, and Head, Human Immunology and Cancer Program University of Tennessee Medical Center/Knoxville, 1924 Alco Highway, Knoxville, Tennessee 37920.** *The University of Ter nessee is an affirmative action, Title IX/Section 504 employer.*

(NW3079)A

NEUROSCIENCES FACULTY POSITION UCR/UCLA PROGRAM IN BIOMEDICAL SCIENCES

The UCR/UCLA Program in Biomedical Sciences, which offers an accelerated M.D. curriculum jointly between the University of California, Riverside (UCR), and the University of California, Los Angeles (UCLA), School of Medicine, has a tenure-track faculty opening in the Division of Biomedical Sciences at UCR available July 1, 1989. The prospective level of appointment for candidates who must possess the Ph.D. or M.D. degree may be at the Assistant, Associate or Full Professor. Primary teaching responsibilities will be in a 10-week course in neurosciences taught to 24 first-year medical students. Prior medical school teaching experience is strongly preferred. Also the successful candidate should actively participate in graduate student training. Junior applicants should have postdoctoral research experience, should be able to develop a strong independent research program and must possess a strong commitment to teaching. Senior applicants should have a clearly demonstrable record of research productivity, success in obtaining extramural funding and demonstrated teaching effectiveness. We would particularly welcome applications from individuals with research expertise in cellular or molecular neurobiology. The University of California is an Affirmative Action/Equal Opportunity Employer. The deadline for applications and nominations is February 28, 1989. Applicants should send a curriculum vitae, bibliography, two to three representative reprints, a statement of present and future research goals, and the names of three references.

Mary Ann Baker, Chair
Neurosciences Search Committee
Division of Biomedical Sciences
University of California, Riverside
Riverside, California 92521-0121

(NW3055)A

CELL IMAGING SCIENTISTS

Postdoctoral positions available for imaging research on new laboratory systems at the Mayo Foundations (S. R. Taylor) and UCLA School of Medicine (K. P. Roos). Applicants should have a career interest in bioengineering and research interests that complement those of existing members of a group studying contraction and excitation-contraction coupling in isolated living muscle cells. The Mayo site is the administrative base, and has a primary interest in acquisition and analysis of data from skeletal muscle (S. R. Taylor). The Los Angeles site has the same analytical tools and a primary interest in cardiac muscle (K. P. Roos). Possible areas of interest for an applicant include: (i) development of mathematical methods for image processing and analysis. (ii) artificial intelligence, (iii) pattern recognition, and (iv) hardware design for high-speed CCD acquisition of bright field or low-light level intensified images. Skill in communication with scientists from different disciplines as well as the ability to work independently major factors. Send application letter, résumé, and addresses and telephone numbers of three references to: **S. R. Taylor, PhD., Mayo Foundation, 711D Guggenheim Building, Rochester, MN 55905.**

An Equal Opportunity/Affirmative Action Institution. (NW3087)A

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COLLAGEN BIOCHEMIST/ENZYMOLOGIST

RESEARCH FELLOW

A$31003 pa — A$38045 pa

Division of Fisheries Tasmania, Australia

The Program: The Seafood Technology Section of the Division of Fisheries carries out a program of work in research, development and liaison concerning the properties, processes and storage characteristics of Australian seafoods.
The appointee will undertake research into the mechanisms of post mortem change in collagen and in softening of fish flesh, isolate and characterize enzymes responsible for the collagenolytic activity, locate these enzymes in the intact tissue and explore potential uses for them in seafood and food processing.
Applicants should hold a PhD or equivalent qualification in one of the following; biochemistry, enzymology, pathology or immunology. Post-graduate research experience in one of the above with a knowledge of collagen biochemistry, its metabolism and degradation by enzymes is essential. Experience in extraction, isolation and purification of collagen fractions and collagenases or lysosomal enzymes is necessary together with a demonstrated ability to plan and implement goal-oriented research. A knowledge of muscle structure and function, post mortem biochemistry and experience with electrophoretic techniques and electron microscopy is advantageous.

Conditions: Appointment will be for a 3 year term to 31/12/91 and Australian Government Superannuation benefits are available. The appointee will be located at the CSIRO Tasmanian Regional Laboratory, in Hobart in the first instance but may be required to transfer to the Division's Laboratory, in Cleveland near Brisbane in the future.

More Information: Further information and a copy of the job description and selection criteria can be obtained from Mr. A. Bremner on Aust. (002) 201444 or Fax Number (002) 201419 or (002) 240530.

Applications should be submitted by the 27th of January 1989 and quote reference number FC 88/40. They should be framed against the selection criteria and should state relevant personal particulars including details of qualifications and experience. Applicants should nominate at least two professional referees and address their applications to:

The Chief
CSIRO Division of Fisheries
Marine Laboratories
GPO Box 1538
Hobart Tasmania AUSTRALIA 7001

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W5718)A

CENTRAL MANCHESTER HEALTH AUTHORITY
SAINT MARY'S HOSPITAL
NORTH WEST REGIONAL IMMUNOGENETICS LABORATORY

GRADUATE SCIENTIST

A post is available at Scientific Officer level in the Immunogenetics Laboratory for two years to carry out research into the use of DNA probes for donor-recipient matching in human bone-marrow transplantation, funded by the North West Regional Health Authority. Applicants should be science graduates with an interest or experience in the use of DNA probes in molecular genetic analysis and with some knowledge of Immunology. Starting salary is up to £8,102 per annum, depending on qualifications.

Informal enquiries to Dr G M Taylor on (061) 276 6472.

Application forms and job descriptions available from the Unit Personnel Department, Saint Mary's Hospital, Whitworth Park, Manchester on (061) 276 6205, between 5.00 pm and 8.30 am, (Ansaphone). CLOSING DATE: 20 January 1989. (8006)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH
DIVISION OF BIOTECHNOLOGY

LABORATORY TECHNICIAN — CANCER RESEARCH CAMPAIGN

Applications are invited for a technician to be appointed within the Medical Physics Technician scale in the salary range £4,711-£7,384. Minimum qualifications required are two science 'A' levels or 4 'O' levels and 2 years relevant technical experience. Candidates with a higher qualification will have an advantage on salary scales. The appointment will be for two years in the first instance.

Further information available from Dr R. Sherwood.

Application forms may be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG, tel: (0980) 610391, ext. 240, to whom completed forms should be returned by 20th January 1989, quoting Post No. 0970. (7985)A

Universität Heidelberg

In der **Fakultät für BIOLOGIE** ist zum 01. April 1991 eine

C4 — PROFESSUR

für **Allgemeine Botanik** (Nachfolge Prof. Dr. M. Bopp)

wiederzubesetzen.

Bewerber/innen sollten auf aktuellen Forschungsgebieten arbeiten. Es wird erwartet, daß sich der/die zukünftige Stelleninhaber/-in am Lehrprogramm des Grund- und Hauptstudiums beteiligt.

Bewerbungen mit den üblichen Unterlagen sind bis zum **15.04. 1989** an den Dekan der Fakultät für Biologie, Universität Heidelberg, Im Neuenheimer Feld 234, 6900 Heidelberg, zu richten. (W5705)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY
ST JAMES'S HOSPITAL

LECTURER (BIOCHEMISTRY)

Applications are invited for the above post available from 1 April 1989.

Salary is on the non-clinical academic scale for Lecturers (Grade B) (£15,105-£19,310) according to qualifications and relevant experience.

The applications of biochemistry in the speciality are extremely broad and the applicant will have opportunities for research in almost any aspect of biochemistry. The successful applicant will also have teaching opportunities, amounting to two or three sessions per week, in both the Biochemistry Department and the University Department of Obstetrics and Gynaecology. The Department has facilities for most biochemical techniques, including radio-immunoassay, HPLC and electrophoresis.

Informal inquiries about the post may be made to Professor R J Lilford, Department of Obstetrics and Gynaecology, St. James's Hospital, Leeds LS9 7TF, (tel. (0532) 433144 Ext. 5872/4524, evenings (0532) 663227, or (0860) 629 752).

Application forms and further particulars may be obtained from and completed applications forwarded to the **Registrar, the University, Leeds LS2 9TJ, (tel (0532) 333965 — direct line), quoting reference number 99/6.** Closing date for applications 26 January 1989. (7984)A

MRC AIDS DIRECTED PROGRAMME
University of Edinburgh

RESEARCH FELLOW

A postdoctoral scientist is required to join a group working on cellular and molecular aspects of Human Immunodeficiency Virus. The vacant position is available for 3 years from 1st January 1989 to study the antiviral effects of substituted oligonucleotides and is funded by an MRC project grant to Dr. T. Brown (Department of Chemistry) and Drs. J.O. Bishop and A. J. Leigh Brown (Department of Genetics).

The appointment will be on the Research Grade 1A scale with a starting salary up to a maximum of £13,870 for a suitably experienced candidate.

Candidates with a background in virology, cell or molecular biology are encouraged to apply, with full C.V. to:
The Departmental Superintendent, University of Edinburgh, Department of Genetics, West Mains Road, Edinburgh EH9 3JN

Further information may be obtained from Dr J.O. Bishop (031 667 1081 ext. 3553) or Dr A.J. Leigh Brown (031 667 1081 ext. 3551). *Please quote reference no. 5619* (7976)A

UNIVERSITY OF WARWICK

RESEARCH TECHNICIAN

Department of Biological Sciences

The Department of Biological Sciences has a vacancy in the Animal Molecular Genetics Group, to work with Dr Cahir O'Kane on embryonic neural development in *Drosophila*. Experience of, or interest in genetics or Molecular Biology would be an advantage.

Salary on Technician Grade 3 scale: £6,636-£7,828 pa.

Application forms from the **Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523685) quoting Ref No 20/T/88/14** (please mark clearly on envelope). Closing date 19 January 1989.

AN EQUAL OPPORTUNITIES EMPLOYER (7982)A

UNITED MEDICAL AND DENTAL SCHOOLS
of Guy's & St Thomas's Hospitals

LABORATORY SCIENTIFIC OFFICER

Applications are invited for the post of Laboratory Scientific Officer in the Division of Medicine at our Guy's Campus (close London Bridge BR and London Transport) to work on a Medical Research Council-funded project on leukocyte/endothelial cell interactions. The successful applicant will be involved in tissue culture and the generation of monoclonal antibodies.

This post is tenable for one year in the first instance. Salary scale: £5,867 to £9,435 per annum (currently under review) plus £1,267 p.a. London Weighting Allowance, according to age, experience and qualifications.

For further information, please telephone Mr Parkes, Chief LSO, on 01-407 7600 x 3527. To apply, please write with full CV and names and addresses of two referees to:— **The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH,** quoting Reference No. G/MED/264. Closing date: 31st January 1989. (7988)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

RESEARCH ASSISTANT

DEPARTMENT OF PHYSIOLOGY

Applications are invited for the above post to work on the cellular actions of anaesthetics. The post is funded by the Wellcome Trust for three years. Candidates should hold a good honours degree in a relevant biological subject.

Salary will be on the Research Scale 1B plus £1,650 London Allowance and there will be the opportunity to register for a higher degree.

Further details can be obtained from Dr. G. Pocock or Dr. C. Richards 01-794 0500, extn. 4313/4315. Application by c.v. (with names and addresses of two referees) should be sent to the **School Office, Room G650, R.F.H.S.M., Rowland Hill Street, London NW3 2PF** by *20 January 1989,* from which full job descriptions are available (tel. 01-794 0500, extn. 4262). Please quote ref. *GP/CD.* (7980)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

RESEARCH ASSISTANT

Department of Medicine I
(Dermatology Laboratories)

Required for studies on skin microcirculation, lymph vessels and pathophysiology of oedema. The work involves imaging of human skin blood and lymph vessels by Fluorescence Video-microscopy and studies of interstitial fluid in lymphoedema and skin disease. The position is for 1-3 years and could lead to a PhD. It is under the joint supervision of Dr P S Mortimer (Senior Lecturer in Medicine/Consultant Dermatologist) and Dr J R Levick (Senior Lecturer in Physiology). Applicants should have a degree in Biological Science or Bio-engineering. Starting salary £8,675 in scale 1B for Research and Analogous Staff plus £1,450 London Allowance per annum. Further particulars and an application form from the **Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Tel 01-672 9944 x 56019.** Closing date 26 January 1989. Quote reference 182/88. (7977)A

MEDICAL MOLECULAR BIOLOGY UNIT
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY AND MIDDLESEX SCHOOL OF MEDICINE

Applications are invited for the post of

Chief Technician

in the above Unit. This is a permanent post involving responsibility for the day to day running of a Unit containing thirty scientists applying molecular techniques to the study of human disease. Duties will include responsibility for ordering, organization of equipment maintenance and repair and supervision of other technical staff as well as some scientific research. Candidates should have previous technical experience in the biological sciences. Salary on the TG5 scale up to £9,549 (plus London Allowance £1,750) depending on age and experience. Applications to **Dr. D. S. Latchman, Medical Molecular Biology Unit, The Windeyer Building, Cleveland Street, London W1P. Telephone No. 01-636 8333 xt. 3100.** *Equal Opportunities Employer.* Closing date: three weeks after appearance of ad. (7990)A

Max-Planck-Institut für Molekulare Genetik
Otto-Warburg-Laboratory
for Biochemistry and Molecular Biology

POSTDOCTORAL POSITIONS

are available to work on

1) RNA processing in trypanosomes and HeLa cells, and the development of *in vitro* reconstitution and processing systems (Dr. Albrecht Bindereif),

or

2) structure and function of eucaryotic tissue-specific and developmentally regulated transcription factors (Dr. Claus Scheidereit).

Applicants should send curriculum vitae, summary of previous research experience, and names of two referees to

Dr. Albrecht Bindereif or **Dr. Claus Scheidereit**
Max-Planck-Institut für Molekulare Genetik
Ihnestr. 73
D-1000 Berlin 33
FRG (W5704)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE
ACADEMIC UNIT OF DIABETES AND ENDOCRINOLOGY AT THE WHITTINGTON HOSPITAL

RESEARCH ASSISTANT

A research assistant post is available in this Unit based at the Whittington Hospital, Highgate, London N19. This exciting new Unit is working on areas covering the complications of diabetes, and the hormonal control of sodium balance. The successful applicant should have a science degree or equivalent and should be familiar with routine biochemical techniques, and have an enquiring approach and good organisational skills. Previous experience with radioimmunoassay and familiarity with techniques of platelet handling and methodology, and with computing would be an advantage. Salary Scale 1B £8,675 to £11,680 (+ LW £1,650) according to experience. For further information contact: Dr. John S. Yudkin MD FRCP or Dr. Peter J.A. Moult MD FRCP on 01-272 3070 ext 4189. Please submit applications and CV to:— **Dr. J S Yudkin or Dr. P J A Moult, Academic Unit of Diabetes and Endocrinology, Whittington Hospital, Archway Road, London N19 5NF.**

Closing date 26 January 1989.

Equal Opportunities Employer (8014)A

DEPARTMENT OF ZOOLOGY
UNIVERSITY OF HAWAII
PROFESSOR IN MOLECULAR BIOLOGY

We seek a senior faculty member with research interests in the biology of nucleic acids, proteins, carbohydrates or lipids. Applicants should: (i) have an active research program focussed on basic biological questions at the molecular level such as gene action and regulation, molecular evolution, or adaptive metabolism, and (ii) have an interest in enriching our undergraduate program in the area of molecular biology. The successful applicant will be selected on the basis of leadership potential to serve as Department Chair, outstanding research accomplishment demonstrating sustained program growth and development, and commitment to teaching excellence. We are particularly interested in individuals who enjoy interacting with biologists of diverse research interests, and whose research will thrive in the unique environment of the Hawaiian archipelago. The position is available as early as 1 August 1989.

Send curriculum vitae, summary of research and teaching experience and philosophy, reprints, and the names and addresses of three references to: **Dr. Robert A. Kinzie III, Chair, Search Committee, Department of Zoology, University of Hawaii, Honolulu, HI 96822.** Deadline for applications is 1 February 1989.

The University of Hawaii is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply. (NW3056)A

THE LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)

MEDICAL MICROBIOLOGIST

We are looking for a Medical Microbiologist to join the Department of Clinical Sciences at the London School of Hygiene and Tropical Medicine, Keppel Street, London W1. The appointment will be made at the level of Clinical Lecturer/Senior Lecturer depending on experience.

The Department of Clinical Sciences was formed by the amalgamation of the department of Medical Microbiology, Clinical Tropical Medicine and Clinical Nutrition.

The post will involve full participation in the teaching commitments of the department, particularly the MSc in Medical Microbiology and the supervision of PhD students. Candidates will be expected to have an established research record and will participate in departmental research activities.

Salaries will be on the universities scale for clinical lecturers/senior lecturers — £13,470-£23,610 or £21,825-£30,800 plus £930 London Weighting. Please send completed application with curriculum vitae and the names of three referees to the **Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.** Closing date for applications 10 February 1989. (7979)A

PLANT MOLECULAR BIOLOGIST
McGill University

The Department of Biology invites applications for a tenure-track position in plant molecular biology. The successful candidate will be expected to develop a strong research program and to contribute to graduate and undergraduate teaching. The department has excellent facilities for plant growth and tissue culture.

The starting date will be on or before September 1, 1989. Rank and salary will be comensurate with experience.

Send curriculum vitae, statement of research interest, representative reprints, and have three letters of reference forwarded to:

Dr. Barid Mukherjee, Chairman, Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada H3A 1B1. *Deadline for applications February 15, 1989.* (NW2994)A

POSTDOCTORAL POSITION

Position presently available for candidate with background in one or more of the following areas: pharmacology, biochemistry, cell biology, immunology or virology. Candidate will participate in research on the evaluation of anti-viral drugs in HIV *in vitro* and *in vivo* systems (nonhuman primates). Studies will be supervised by Dr. Jorg Eichberg. Appointments are for one year periods up to three years. Annual salaries range from $21,070 to $29,265 dependent upon previous postdoctoral experience. Send curriculum vitae with names and telephone numbers of three references to **Personnel Director (906), Southwest Foundation for Biomedical Research, P.O. Box 28147, San Antonio, TX 78284.** Equal opportunity employer m/f. (NW3060)A

RESEARCH ASSOCIATE IN BRYOLOGY
Farlow Herbarium
Harvard University

Applications are invited from systematists with Ph.D. in Botany who are conducting research in Bryology. Training and interests should include new or promising methodologies, techniques, or philosophies in systematic and evolutionary research. Primary responsibility will be to conduct an active research program in Bryology, as well as to participate in administration and curation activities of the Farlow Herbarium bryophyte collection.

Appointment to be annual with possibility of up to five years. Applications including a curriculum vitae, statement of research interests, reprints of recent publications and three letters of reference should be sent by March 1, 1989, to:

Dr. Donald H. Pfister
Harvard University Herbaria
22 Divinity Avenue
Cambridge, Ma. 02138

Harvard University is an Affirmative Action/Equal Opportunity Employer. (NW3081)A

ROYAL HOLLOWAY AND BEDFORD
NEW COLLEGE
University of London

LECTURESHIP IN GEOLOGY

Applications are invited for the post of Lecturer in Structural Geology/ Seismic Stratigraphy to work with a vigorous team researching into basin evolution. Field-based experience, mathematical skills and computer modelling abilities are fundamental requirements. Depending on qualifications and experience the appointment will be made on either Lecturer Grade A (£10,910-£16,150 inclusive) or Lecturer Grade B (£16,755-£20,960 inclusive).

Further details and application forms may be obtained from **Mrs F Maynard, Personnel Office, Royal Holloway and Bedford New College, Figham Hill, Egham, Surrey, TW20 0EX. Telephone Egham 34455 extension 3027.** Closing date for receipt of applications is 27th January 1989. (7995)A

Physical Oceanographer
Search Extended

Applications are invited for a tenure track, assistant or beginning associate professorship; Phd required.

We seek a process oriented oceanographer interested in using modern instrumentation in field studies of coastal and estuarine dynamics, an individual who can take advantage of diverse funding opportunities including state and local sources.

Expected is a strong commitment to teaching, advising graduate students, and developing a significant research program in an interdisciplinary setting, including the application of science to solving environmental and/or societal problems. Salary commensurate with experience.

Please send curriculum vitae, summary of research interests, and names of three referees by February 15, 1989 to **Dr. M.J. Bowman, Marine Sciences Research Center, SUNY Stony Brook, Stony Brook, NY 11794-5000.**

SUNY Stony Brook is an affirmative action/equal opportunity educator and employer. AK# 347. (NW3068)A

ASSISTANT PROFESSOR

Theoretical Ecologist or Theoretical Population Biologist

Applications are invited for a tenure track Assistant Professorship *beginning August, 1989.* Teaching responsibilities will include a theoretical ecology or population biology course and participation in undergraduate offerings. The University of Wisconsin-Madison is an Affirmative Action, Equal Opportunity Employer and encourages women and minorities to apply.

Please submit curriculum vitae, copies of publications, description of research and teaching interests, and have three letters of recommendation sent by 1 March 1989 to:

Chair, Population Biologist Search Committee
Department of Zoology
University of Wisconsin-Madison
Madison, Wisconsin 53706, USA
608-262-1087 (NW3085)A

University of Edinburgh
The Duke of Edinburgh Chair of Cardiology

The University Court invites applications for the Duke of Edinburgh Chair of Cardiology which is funded by the British Heart Foundation and becomes vacant on 1st July 1989, on the retirement of Professor Michael Oliver. The University seeks to appoint someone with wide experience of cardiology who has made significant contributions to knowledge of cardiovascular disease and who is capable of advancing understanding of the subject and of providing leadership for the staff of the Cardiovascular Research Unit. The appointee will also be expected to teach undergraduate and postgraduate students. He/she need not necessarily be medically qualified, but a clinician would be expected to contribute to the clinical service in the Royal Infirmary. Further particulars may be obtained from the **Secretary to the University, University of Edinburgh, Personnel Office, 63 South Bridge, Edinburgh EH1 1LS,** with whom applications (15 copies), including the names of three referees, should be lodged not later than 3rd March 1989. Overseas applicants need only supply one copy of application. *Please quote reference No. 60/88* (8007)A

HARVARD MEDICAL SCHOOL
MASSACHUSETTS GENERAL HOSPITAL

POSTDOCTORAL RESEARCH SCIENTIST
MOLECULAR AND DEVELOPMENTAL NEUROBIOLOGY

Postdoctoral scientist position available as of the spring of 1989 to study gene regulation during the development of neurons. Ongoing efforts include the identification of genes related to neuronal differentiation and plasticity, the study of their transcriptional control, and the analysis of their function by perturbation *in vitro* and in transgenic mice.

Interested persons should send curriculum vitae to:

Dr. Mark C. Fishman
Howard Hughes Medical Institute
Wellman 4
Massachusetts General Hospital
Boston, MA 02114 (NW3062)A

UNIVERSITY OF CAMBRIDGE
INSTITUTE OF ASTRONOMY

FACULTY POSITION

A post is available for an astronomer whose main duties would be research and the supervision of graduate students. The appointment is at the level of Senior Assistant in Research, and will be for an initial period of five years with the possibility of reappointment and upgrading. Although some preference may be given to candidates with theoretical interests, applications are welcome from astronomers whose research relates to any part of the Institute's programme.

The stipend is on the age-related scale £11,680 (age 27) to £14,500 (age 32 or above). Further information about the duties and conditions of the appointment may be obtained from the **Secretary of the Appointments Committee, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA,** to whom candidates should send their applications (ten copies — overseas candidates need only send one copy) including a curriculum vitae and names of not more than three referees, so as to reach him not later than 17 February 1989. (8010)A

Childrens Hospital of Los Angeles

The Division of Research Immunology & Bone Marrow Transplantation of the Childrens Hospital of Los Angeles wishes to recruit individuals for a junior faculty position. The successful candidate will be expected to establish an independent research program with external funding and to engage in the clinical bone marrow transplantation program of the Division. Previous clinical experience in the areas of Hematology/ Oncology, Immunology and/or Infectious Disease is required. Research expertise in the area of oncology, molecular biology and/or immunology is preferred. Candidates should send their curriculum vitae, names of three references and a brief description of their research interests to:

Robertson Parkman, M.D.
Head, Division of Research Immunology
& Bone Marrow Transplantation
Childrens Hospital of Los Angeles
4650 Sunset Blvd.
Los Angeles, CA. 90027

The Childrens Hospital of Los Angeles and the University of Southern California, School of Medicine are Equal Opportunity Employers (NW3059)A

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the widest international selection of jobs in science — EVERY WEEK

Clinical Electrophysiologist

The Department of Otorhinolaryngology and Human Communication, University of Pennsylvania School of Medicine seeks a Ph.D clinical neurophysiologist. The applicant must have demonstrated expertise, at least five years experience and refereed publications in multimodality evoked potentials, with specialized expertise in intraoperative and intensive care monitoring of evoked potentials and quantitative EEG. Expertise in topographic brain electrical activity mapping is required. Contact: **Daniel M. Schwartz, Ph.D Division of BioelectroDiagnosis and Neural Monitoring, University of Pennsylvania Medical Center, 3400 Spruce Street, Philadelphia, Pa.**

The University of Pennsylvania is an Equal Opportunity Employer. (NW3069)A

DIRECTOR, RADIOIMMUNOASSAY CORE,

Diabetes Endocrinology Research Center, University of Washington Division of Endocrinology/Metabolism, Seattle, Washington. This is a research faculty position with rank dependent upon experience. The applicant should have a Ph.D. degree and a strong background in peptide chemistry and development of hormone radioimmunoassays. The major responsibility will be directing the Core laboratory which processes routine radioimmunoassays, and development of new assays as required by the Affiliate Investigators of the Center. Approximately 50% of time will be available for independent research, and independent grant support is highly desirable. Training, expertise, interest and capability in diabetes, endocrinology and metabolism research is desired. For further information please contact: **Dr. Leslie J. Klaff, Acting Director, Radioimmunoassay Core, Diabetes Endocrinology Research Center, Quarters 8, Pacific Medical Center, 1200-12th Avenue South, Seattle, WA 98144; Telephone: 206-543-6481.**

The University of Washington is an Equal Opportunity Employer. (NW3088)A

The Queen's University of Belfast

CHAIR OF EARTH RESOURCES

e University intends to develop present Department of Geo- y as a school of Earth Resour- , offering a strong geology core, directing its programmes ticularly to applied concerns ing from the mineral and rocarbon industries and explo- ion and economic utilisation of earth's resources. The Univer- wishes to appoint a Professor will initiate teaching grammes; develop research, ticularly in applied areas and end links with industry and loration companies. The per- appointed would initially become the Director of the School and have the opportunity to lead new developments in geology and earth resources in Northern Ireland. A significant expansion in staff is expected to accompany this appointment.

Salary will be within the professorial range with contributory pension rights under USS.

The appointment will be from 1 October 1989 or such other date as may be agreed.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: 27 February 1989 (Please quote Ref. 89/). The University is an Equal Opportunity employer. FEA Registration No. 575. (8026)A

POSTDOCTORAL POSITIONS BIOLOGICAL THEORY

Protein structure and function; DNA structure and function; neural net theory. Research potential more important than specific training.

For information, contact **Charles DeLisi, Chair, Department of Biomathematical Sciences, The Mount Sinai School of Medicine, Box 1023, One Gustave L. Levy Place, New York, NY 10029 6574.**

An Equal Opportunity Employer. (NW3051)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

MOLECULAR BIOLOGY

Applications are invited from suitably experienced pre-or post-doctoral scientists to join a group involved in studying the molecular genetics of atypical mycobacteria, particularly in relation to Crohin's disease in humans and Johne's disease of cattle. The work involves the use of DNA sequence from a newly, identified mycobacterial insertional element and PCR amplification, to assay disease and environmental samples for specific slow growing atypical mycobacteria.

The appointment is for 1 year initially with the possibility of extension, with a start my salary upto £13,130 inclusion of London Weighting. Applications together with the names and addresses of 2 referees to **Professor John Hermon-Taylor, Department of Surgery, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, tel 01-767 7631,** from whom further particulars may be obtained. Closing date 20 January 1989. Please quote reference 195/88. (7991)A

Cell Biologist
Biophysicist
Structural Biologist
Molecular Biologist

The Pennsylvania Muscle Institute and the University of Pennsylvania School of Medicine seek outstanding candidates for two tenure track positions in the area of fundamental cardiovascular or motility research. One position will be in the field of molecular genetics of contractile proteins.

The other position is in the broad area of cell biology, biophysics and/or structural biology of muscle. These positions are primarily aimed at, but not restricted to, the Assistant Professor level. Appointment will be made in the department appropriate to academic interest.

Applicants should send CV and research statements and the names of three references to: **Yale E. Goldman, M.D., Ph.D., Director of Pennsylvania Muscle Institute, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6085.**

Equal Opportunity/Affirmative Action Employer. (NW3052)A

The Medical College of St. Bartholomew's Hospital

POST-DOCTORAL RESEARCH FELLOW

£11,517-£17,370 p.a.

A Research Fellow is required in the Department of Gastroenterology, St. Bartholomew's Hospital to work with a group of medical and basic scientists on the pathogenesis of some intestinal infections, particularly giardiasis and amoebiasis. The successful applicant would be involved in ongoing research, including the development of DNA probes for genetic analysis of parasites and for diagnosis. The group is also attempting to identify genes encoding for immunodominant parasite antigens. Previous experience in cell culture, monoclonal antibody production and molecular biological techniques is desirable.

Enquiries to Dr. M.J.G. Farthing to whom application should be made in the form of a curriculum vitae with the names of 3 referees to **St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE. Tel: 01-601 8508/9/10.**

Closing date: 28th January. (8003)A

POSTDOCTORAL POSITION

Available in spring 1989 at 120,000 FF/year for research on molecular and/or cell biology of neuronal proteins and brain development. Send resume, publications and reference letters to **J. Nunez, Unité INSERM 282, Hopital Henri Mondor, 94010 Créteil France.** (W5698)A

Yale UNIVERSITY

The McKnight Foundation

Molecular Genetics of the Flowering Process

Graduate and Postdoctoral Fellowships

An interdisciplinary research program at Yale University sponsored by The McKnight Foundation.

Applications are invited for Graduate and Postdoctoral Fellowships to join an interdisciplinary research program undertaken by ten faculty members in three departments at Yale University (Biology, Molecular Biophysics & Biochemistry, and Forestry & Environmental Studies).

Ongoing projects include the following:

- genetic analysis of cell-cell interactions in floral structures
- mutational and molecular fine-structure analysis of floral genes
- ectopic expression of growth regulators in the flowering process

The training program also includes technical workshops, seminar programs, and extensive collaborative work. Graduate applicants must apply to the Director of Graduate Studies, Department of Biology, P.O. Box 6666, Yale University, New Haven, CT 06511 U.S.A., Telephone: (203) 432-3538 by March 1, 1989. Postdoctoral applicants should apply directly to the Project Coordinator with CV, three letters of reference, and a research interest statement. Postdoctoral fellows will be encouraged to develop independent collaborative projects on flowering.

For further information, contact Prof. Ian Sussex (Project Coordinator), Yale Biology Department, P.O. Box 6666, New Haven, CT 06511 U.S.A.

Yale University is an Affirmative Action/Equal Opportunity Employer.

(NW3070)E

RESEARCH FELLOWSHIPS IN NUTRITION FOR MEDICAL STUDENTS THE ROCKEFELLER UNIVERSITY

A program for one year research fellowships has been made available by the Pew Charitable Trusts. Medical students will be selected to join the Pew Center of Nutritional Excellence at Rockefeller University in New York City. The core activity of fellows will be investigative work in basic biomedical laboratories in Rockefeller University on projects relevant to human nutrition. Applicants should currently be in the junior year of any medical school in the U.S.A. A generous stipend and housing allowance will be offered to those selected for the fellowship year to begin July 1, 1989.

Applications must be completed no later than February 1, 1989 to allow for full consideration. A small number of applicants will be invited to New York City for interviews in March, 1989 and final selection will be made early in April. For application forms and further details write to:

Dr. Jules Hirsch
The Rockefeller University
1230 York Avenue
New York, New York 10021-6399

(NW3075)E

ROYAL SOCIETY OF EDINBURGH

BP RESEARCH FELLOWSHIPS

The next round of the prestigious post-doctoral BP Research Fellowships will be advertised shortly. The Fellowships are for research in: Mechanical Engineering; Solid State Sciences; Information Technology; Organic Chemistry (except natural product and biological chemistry); Geological Sciences. Applications are available from the **Executive Secretary, Royal Society of Edinburgh, 22-24 George Street, Edinburgh EH2 2PQ.**

The BP Research Fellowships were created in 1988 and launched at a reception, held at the Royal Society of Edinburgh in July attended by the Secretary of State for Scotland. Two BP Research Fellows were appointed and have taken up post; one at the University of Stirling and one at the University of Glasgow.

Dr Roger Watt came from Cambridge to the University of Stirling, where a new Centre for Cognitive and Computational Neuroscience is being set up. Dr Watt feels his BP Fellowship has helped a great deal in setting up this centre and explains its work.

"The centre will bring together mathematicians, computer scientists and psychologists with a common interest in the performance of new parallel computer architectures modelled on neural networks. We are principally concerned with studying vision, but by machine and people. Vision is a particularly useful task to study because it is something that humans are very good at, but which has proved immensely difficult even to begin to emulate in standard computer systems."

Dr Peter Haughton's BP Research Fellowship is to carry out research at the University of Glasgow on the 'Evolution of late-orogenic basins in the Betic-Rifian Orogeny of southern Spain and northern Morocco'.

The Royal Society of Edinburgh hopes to appoint two, or possibly three, BP Research Fellows to begin in October 1989.

(8005)E

The Unit 42 of the French National Institute of Health and Medical Research (INSERM U 42) is offering a

POST DOCTORAL RESEARCH FELLOWSHIP

for 1 year with the possibility of extension and further permanent position.

Applicants should possess a PhD or equivalent doctoral degree and have experience in

MOLECULAR BIOLOGY

with emphasis on CDNA/gDNA library, cloning and sequencing.

The major research project concerns the characterization of antigens of *Toxoplasma gondii* or *Pneumocystis carinii*. Experience in parsitology is not essential. Salary range is 8,00–14,000FF

Applications (with full C.V. and the names of 2 referees) and informal inquiries to:

Professor Daniel CAMUS
INSERM U 42
369, rue Jules Guesde
59650-Villeneuve d'Ascq
FRANCE

as soon as possible

(W5710)E

EMBO
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS
in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from **Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G.** (W5706)E

UNIVERSITY OF LEICESTER
DEPARTMENT OF GENETICS
Postdoctoral Research Fellowship

Applications are invited immediately for an 18 month appointment funded by Action Research for the Crippled Child. (A further period of support will be sought for a suitable successful candidate). The project concerns the isolation of collagen cDNA clones for use in the investigation of inherited human connective tissue disorders. The successful candidate will join a group of six and will be experienced in some or all of the following areas: RNA isolation, construction and screening of cDNA libraries, RNase A protection mapping, cell culture, PCR amplification, Southern and Northern blotting, computing.

Applicants should possess a Ph.D. in a relevant subject. Salary, according to age and experience, on scale IA: £9,865 to £15,720. Informal enquiries may be made to Dr. Raymond Dalgleish on (0533) 523425. Applications, including a c.v. and the names of two referees should be sent to:– **Dr. R. Dalgleish, Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH** as soon as possible.
(8017)E

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
MOLECULAR BIOLOGIST
Post-doctoral Fellowship/Pre-doctoral Research Assistantship

Available from January 1989 to work on a 2 year MRC funded project to find new ways to express AIDS virus antigen, in eukaryotic cells. Experience in molecular biology techniques would be an advantage. The successful candidate will join an active research team housed in a very well equipped suite of laboratories in the Department of Medical Microbiology.

Informal enquires can be made by telephoning Dr A Coates on 01-377 7259.

Applications including CV and the names of two referees should be sent to:

Miss J T Lewis
Assistant Secretary
The London Hospital Medical College
Turner Street
London E1 2AD

within 2 weeks of the date of this advertisement. (7986)E

POSTDOCTORAL FELLOW
ICRF
CLINICAL ONCOLOGY UNIT
INSTITUTE OF MOLECULAR MEDICINE
JOHN RADCLIFFE HOSPITAL, OXFORD

Applications are invited for a three year postdoctoral fellowship in the new ICRF Clinical Oncology Unit Oxford. The Honorary Director of the Unit is Professor Adrian Harris and the Senior Scientist in the laboratory is Dr. Ian Hickson. The laboratories are situated within the Institute of Molecular Medicine and the research interests of the Unit include cloning human DNA repair genes, drug resistance mechanisms and growth factors and their receptors.

Salary will be dependent on age and experience and in the range of £12,500 to £15,324.

Informal enquiries may be made to Professor Harris (0865 741841, Ext. 698) or Dr. Hickson (091 232 5131).

Applications should be made by sending a full CV and names and addresses of three referees to the Mrs P Harwood, Recruitment Officer, Imperial Cancer Research Fund, PO Box Lincoln's Inn Fields, London WC2A 3PX quoting ref 46/R by 31st January 1989.

Smoking is actively discouraged. (7994)E

IMPERIAL
CANCER RESEARCH FUND

STUDENTSHIPS

QUEEN MARY COLLEGE
(University of London)
Department of Chemistry
Postdoctoral Research Assistants and SERC CASE Studentships

Applications are invited for two postdoctoral positions. Both concern the synthesis and characterization of precursors suitable for producing thin films of electronics materials by Chemical Vapour Deposition. Experience in the handling of air-sensitive compounds is essential.

The first, under the direction of Professor D. C. Bradley, F.R.S., is concerned with metalo-organic precursors for III/V systems, and will involve research collaboration of an interdisciplinary nature with the electronics industry.

The second, under the direction of Professor B. J. Aylett, is concerned with volatile precursors for silicon-based systems and their deposition mechanisms, and will involve research collaboration with a leading company in the U.K. and California.

Two SERC-funded CASE awards are also available for suitably-qualified graduates. The work will be in similar areas to those detailed above.

Applications with a C.V. and names of two referees should be sent *before January 31st* to either of those named above at: **Department of Chemistry, Queen Mary College, Mile End Road, London E1 4NS.**
(8000)F

MISCELLANEOUS

ARWIN abets lamarck? everse transcriptase cau- es inheritance of acquired nmunity? Smallpox? Box. o. 7981 c/o 4, Little Essex treet, London WC2R 3LF.
(7981)J

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STUDENTSHIPS continued

DEPARTMENT OF PHARMACOLOGY
UNIVERSITY COLLEGE LONDON

Research Studentships

The Department will have a number of Research Studentships to offer, for work in the following areas:

1) Synaptic, ion channel and smooth muscle pharmacology
2) Central neuropharmacology (pain pathways, epilepsy, Parkinsonism)

and 3) Immunopharmacology (mediators of inflammation).

Applicants should hold, or expect to obtain, a good honours degree in Pharmacology or a related subject, and should write in the first instance to the **Departmental Postgraduate Tutor, Professor D.H. Jenkinson, Department of Pharmacology, University College London, Gower Street, London, WC1E 6BT.** Please enclose a full C.V. giving an indication of your interests, and the names of two academic referees. (8013)F

GRANTS & SCHOLARSHIPS

UNIVERSITY OF LONDON

EDMUND DAVIS SCHOLARSHIP IN MEDICINE, LAW OR ENGINEERING

Applications are invited from suitably qualified graduates of any university for the award of an Edmund Davis Scholarship tenable from October 1989. Under the terms of the benefaction preference will be given to applicants born in the geographical areas formerly known as Northern and Southern Rhodesia.

The Scholarship is awarded for full-time graduate work at a School of the University for an approved course leading to a Higher Degree at the University. The award is tenable for a period not exceeding three years.

Detailed information and application forms may be obtained from the Secretary, Scholarships Committee, University of London, Room 21A, Senate House, Malet Street, London WC1E 7HU, with whom completed applications and references must be lodged by **no later than 1 March 1989.** (7989)H

WORKSHOPS

MICROELECTRODE TECHNIQUES FOR CELL PHYSIOLOGY

The sixth workshop will take place at the Laboratory of the Marine Biological Association, Citadel Hill, Plymouth, UK from September 6th to 20th, 1989. The workshop aims to provide first hand experience in a number of electrophysiological techniques for postgraduate students and postdoctoral workers. The following techniques will be available:

1. **Patch-clamp**
2. **Voltage-clamp**
3. **Ion-sensitive microelectrodes**
4. **Intracellular dye injection**
5. **Fluorescent dye techniques**

The course fee of £950 includes accommodation, meals and tuition. Some bursaries will be available.

For application forms contact: **Dr. D. Ogden, Pharmacology, Kings College London, The Strand, London WC2R 2LS, UK.**

Closing date for applications: 31st March, 1989.

Microelectrode Techniques: The Plymouth Workshop Handbook is available from the Biochemical Society, tel: 0206-46351 (44-206, USA) for £16/$30. (8001)V

SEMINARS & SYMPOSIA

2nd International Symposium on Positive Strand, RNA Viruses

Vienna, Austria, June 26–30, 1989

Satellite meeting: WHO Review on the development of dengue, Japanese encephalitis and other flavivirus vaccines: June 24-25, 1989.

Contact: Dr. F.X. Heinz, Institute of Virology, Kinderspitalgasse 15, A–1095 Vienna, Austria. Tel: 222/431595 Fax: 222/432161 (W5640)M

PROPOSALS

UKCCCR PROGRAMME ON RADIATION AND CHILDHOOD CANCER

CALL FOR RESEARCH PROPOSALS

Grants are available from the United Kingdom Coordinating Committee on Cancer Research (UKCCCR) for research projects primarily in laboratory-based experimental biology with the following objective:

To ascertain whether in the context of exposure to ionising radiations, there exists any special sensitivity or hitherto undiscovered mechanism that would give rise to a higher incidence of childhood cancer, including leukaemia, than would be expected on the basis of current risk estimates.

The five-year programme will be implemented and independently directed by the UKCCCR with funding provided by the Atomic Energy Authority, British Nuclear Fuels and the Central Electricity Generating Board. The UKCCCR has a broad interest in the causation of cancer including leukaemia. This is being explored in relation to radiation through its recently convened Radiation and Cancer Subcommittee, and this programme has resulted from that interest.

The programme is primarily directed towards basic research into the biological mechanisms underlying risks associated with exposure to ionising radiations. However the Subcommittee has been set up with a broader remit, and there will be further discussions exploring questions relating to epidemiology. The Subcommittee will also, therefore, be interested in work in this area.

Further information on how to submit an outline application (not exceeding 1000 words) is available from **Mrs Jean Mossman, UKCCCR Secretariat, Medical Research Council, 20 Park Crescent, London W1N 4AL Tel: 01-636 5422 Ext 353).**

All sections of the scientific community, whether public or private, may apply. (7999)X

COURSES & CONFERENCES

Genetics Short Course
17-28 July 1989

30th Annual Short Course in Medical & Experimental Mammalian Genetics

Conducted by Johns Hopkins University and The Jackson Laboratory at Bar Harbor, Maine. Morning and evening lectures covering the field are supplemented by workshops, tutorials and demonstrations. Inquiries, applications to: **Dr. T. H. Roderick, c/o Training & Education Office, The Jackson Laboratory, 600 Maine Street, Bar Harbor, ME 04609-0800;** or **Dr. V. A. McKusick, 292 Carnegie Bldg., Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21205.** (NW3053)C

UNIVERSITY CLAUDE BERNARD OF LYON AND THE ROYAL POSTGRADUATE MEDICAL SCHOOL OF LONDON

A LABORATORY COURSE FOR THE PREPARATION OF MONOCLONAL ANTIBODIES

to be held at: Faculté de Médecine Alexis Carrel, Université Claude Bernard, LYON, FRANCE.

from April 24th to April 28th, 1989

The course will be held in english with a group of lecturers having practical experience with monoclonal antibodies. Registration fee is 7,500 French Francs. This fee includes the hotel accommodation and lunches during the week.

Letters of application should be sent by March 20th, 1989, to the attention of **Ms Sophie PARMENTIER, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, F-69372 LYON, FRANCE, Tel: (33) 78.01.31.62.**

Organizers: Dr. J.L. McGregor, INSERM U.63, Faculté Alexis Carrel, F-69372 Lyon, France, and Dr Mary A. Ritter, Royal Postgraduate Medical School of London, Hammersmith Hospital, du Cane Rd, London W12 0HS, UK. (W5506)C

ROYAL SOCIETY DISCUSSION MEETING

MARCH 1989

8 and 9 March: 'Molecular chemistry for electronics', organized by Dr P. Day, F.R.S., Professor D.C. Bradley, F.R.S. and Professor D. Bloor. **Speakers:** G.W. Gray, F.R.S., H. Ringsdorf, J.-C. Marchon, W.J. Feast, J.N. Sherwood, R.A. Hann, P. Hodge, D.C. Bradeley, F.R.S., D.J. Cole-Hamilton, J.O. Williams, A. Ledwith, O. Khan and J.S. Miller.

Registration details (no fee for attendance): Miss C.A. Johnson (ref. DMN/CAJ), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. (Tel: 01-839 5561, ext. 278. Telex: 917876). (8025)C

INTERNATIONAL CONFERENCE

STUDIES INVOLVING NUCLEIC ACID RECOGNITION

17th-20th April 1989

Sheffield University, Sheffied, U.K.

An Independent Meeting of the Nucleic Acids and Molecular Biology Group of the Biochemical Society and the Royal Society of Chemistry

3rd GOBIND KHORANA LECTURER MORIO IKEHARA

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Marvin Caruthers	(USA)	David Lilley	(UK)
Jyoti Chattopadhyaya	(Swe)	Steven Neidle	(UK)
Marius Clore	(FGR)	Eiko Ohtsuka	(Japan)
Fritz Eckstein	(FGR)	Simon Phillips	(UK)
Angela Gronenborn	(FGR)	Wocjiech Stec	(Poland)
Claude Hélène	(France)	Dietrich Such	(FGR)
Robert Kaptein	(Netherlands)	Thomas Tullius	(USA)
		Michael Waring (UK)	

The Conference will focus on studies involving recognition between: Nucleic Acids and Nucleic Acids; Nucleic Acids and Proteins; and Nucleic Acids and Small Molecules.

Contributions on these themes are invited either as short oral presentations or as posters.

Attendance limited to 160 active participants. Bursaries available for graduate students. 2nd Circular and Registration Forms from: **Dr. G. Michael Blackburn, Department of Chemistry, The University, Sheffield S3 7HF, UK. Phone: 0742-768555 Ex-4474. Fax 0742-739826. Telex: 547216 UGSHEF.** (8021)C

IDIOTYPE NETWORKS IN BIOLOGY AND MEDICINE

APRIL 17–20 1989 Gennep, The Netherlands

Congress will cover:

Idiotypes in immunology
Idiotypes in regulation
Idiotypes in viral disease (with a special session on AIDS sponsored by BECTON DICKINSON BENELUX)
Idiotypes in bacterial disease (theme sponsored by PRAXIS BIOLOGICS INC., Rochester)
Idiotypes in parasitic disease (theme sponsored by DUPHAR BV, Animal Health Division, Weesp)
Idiotypes and cancer
Idiotypes and autoimmunity (theme sponsored by IDEC, Pharmaceuticals Corporation, La Jolla)

Invited Lectures by:

J. Bach	S. Ferrone	F. Melchers
M. de Baets	M. Fougereau	A. Nisonoff
C. Bona	M. Greene	A. Rees
N. Bos	D. Harlyn	P. Schuber
H. Bunschoten	G. Hoffmann	K. Stein
D. Capra	Ch. Janaway	K. Thielemans
P. Cazenave	R. Kennedy	J. Urbain
I. Claassen	H. Köhler	E. Weiler
D. Colley	T. Logtenberg	M. Zanetti
A. Coutinho		

Congress secretariat: **OBU Utrecht Congress Office, PO Box 14214, 3508 SH Utrecht, The Netherlands.**

Telephone: 030 523272 **Telefax: 030 522694**

Organizing Committee: A.D.M.E. Osterhaus, J. Urbain, F.G.C.M. UytdeHaag (W5722)C

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Information may be obtained from the **Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.** (7987)C

continued on page 24

MARINE BIOLOGICAL LABORATORY

1989 Summer Courses

BIOLOGY OF PARASITISM (June 11-August 11) Emphasizing the on-going convergence of molecular biology and immunology with tropical medicine, this course covers recent developments in research on parasites and parasitic disease. Laboratory work focuses on the general biology of parasites as well as applicable methods of molecular biology and immunology. Intended for advanced graduate and post-doctoral students as well as established investigators. Co-Directors: John E. Donelson, University of Iowa School of Medicine, and Carole Long, Hahnemann Medical College.

EMBRYOLOGY: CELL DIFFERENTIATION AND GENE EXPRESSION IN EARLY DEVELOPMENT (June 18-July 29) An advanced level lecture and laboratory course intended for graduate students, postdoctoral fellows, and other research scientists who wish a current treatment of major issues in the experimental analysis of developmental biology of invertebrate and vertebrate embryonic systems, and the diverse intellectual approaches now useful in this field. Director: Eric H. Davidson, California Institute of Technology.

MARINE ECOLOGY: APPLICATIONS OF MOLECULAR BIOLOGY TECHNIQUES AND MOLECULAR PROBES (June 18-July 29) Two three-week modules will provide an intensive experience for graduate, postdoctoral and highly qualified undergraduate students. Director: J. Woodland Hastings, Harvard University.

MICROBIOLOGY: MOLECULAR ASPECTS OF CELLULAR DIVERSITY (June 11-July 27) A six-week laboratory course intended for advanced students and established investigators who seek training in microbiological techniques and experience with a broad range of microbes, emphasizing anaerobes and their cultivation. Co-Directors: Ralph Wolfe, Universtiy of Illinois, and Peter Greenberg, University of Iowa.

NEURAL SYSTEMS AND BEHAVIOR (June 11-July 29) A lecture/laboratory course intended for graduate students, postdoctoral fellows, and established investigators combining state-of-the-art methods of neurobiology with the analysis of animal behavior, representing the interface between these two fields. Director: Thomas Carew, Yale University.

NEUROBIOLOGY (June 11-August 19) A ten-week advanced course for pre- and postdoctoral students providing lectures and laboratory experience in the issues and techniques of contemporary cellular and molecular neurobiology. Director: Arthur Karlin, Columbia University.

PHYSIOLOGY: CELL AND MOLECULAR BIOLOGY (June 11-July 22) A course for graduate students, postdoctoral fellows and established investigators who wish hands on experience with both standard methods to purify and characterize proteins, nucleic acids and other cellular components as well as several advanced techniques used to relate molecular structure to physiological function. The course will feature the importance of marine organisms available at Woods Hole as experimental systems in modern biology, and postcourse research with the staff. Director: Thomas D. Pollard, Johns Hopkins University.

Application Deadline: March 1, 1989

Partial financial aid is available to qualified applicants.

Contact: Admissions Coordinator, Office of Sponsored Programs,
Marine Biological Laboratory,
Woods Hole, MA 02543; (508) 548-3705, ext. 216

(NW3046)C

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BERLIN (WEST) 27 JUNE – 12 JULY 1989

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APPLICATION

The school is specially designed for young scientists: graduate students or Ph.D's. There will be plenty of time for formal and informal discussions. Participation is strictly limited to 80 students. Applications should include a C.V. and specific reasons for wishing to attend the school. A letter of recommendation may be added if such information is thought to be important in facilitating the selection process.

Application should be sent to: **Dr. V.E.A. Russo, Max-Planck-Institut für molekulare Genetik, Ihnestraße 73, D-1000 Berlin 33,** and should arrive no later than **28 February 1989.** Applicants will be informed by 1 May 1989 whether or not they were selected. There are no special application forms. If you wish to present a poster you must give the title in your application.

LOCATION: The school is residential. All participants will be accommodated in a conference center in the peaceful environment of Berlin's forest.

REGISTRATION FEE: none.

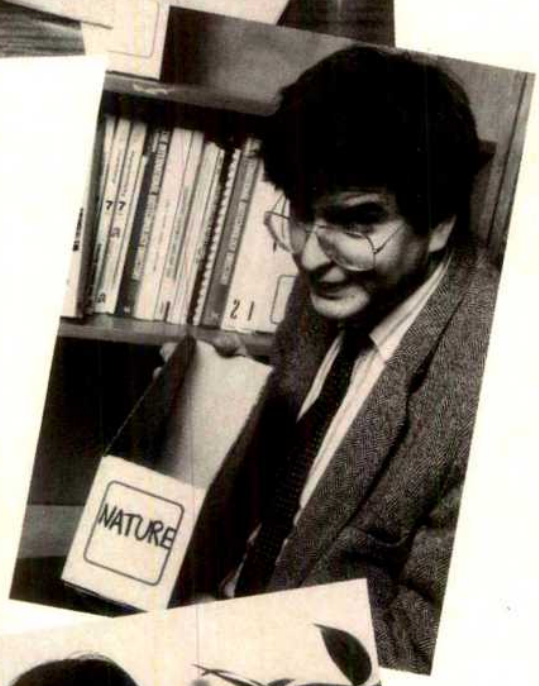
ROOM AND BOARD: DM 1,800. Room and board will be paid upon notice of acceptance to the school.

FELLOWSHIP: Many fellowships are available for young scientists for partial financial support. Write in your application what is the minimum amount of financial support (in DM) you need in order to participate in case you were accepted.

SPONSORSHIP: Max-Planck-Society, Fonds der chemischen Industrie, Bundesministerium für Forschung und Technologie, FEBS, the Senate of Berlin.

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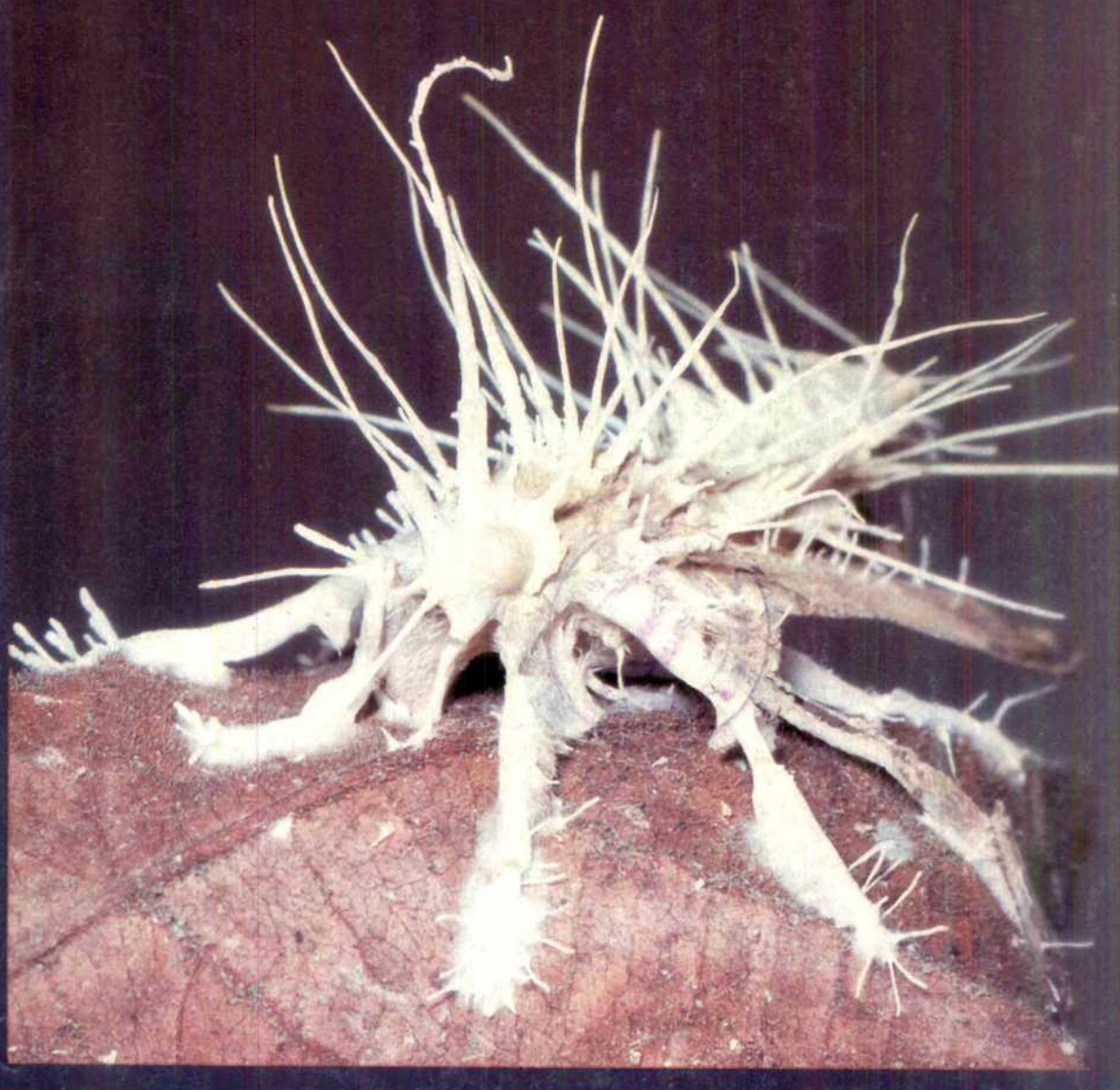
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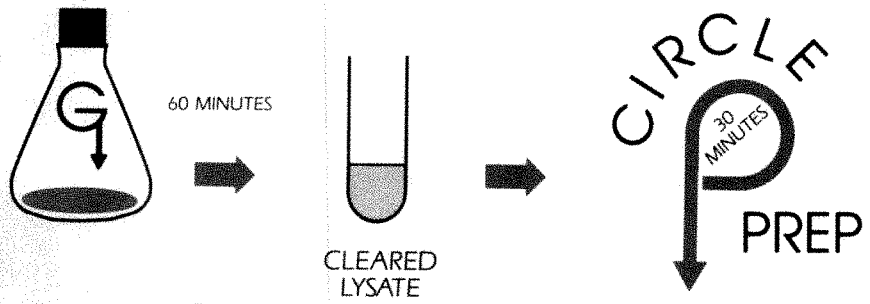
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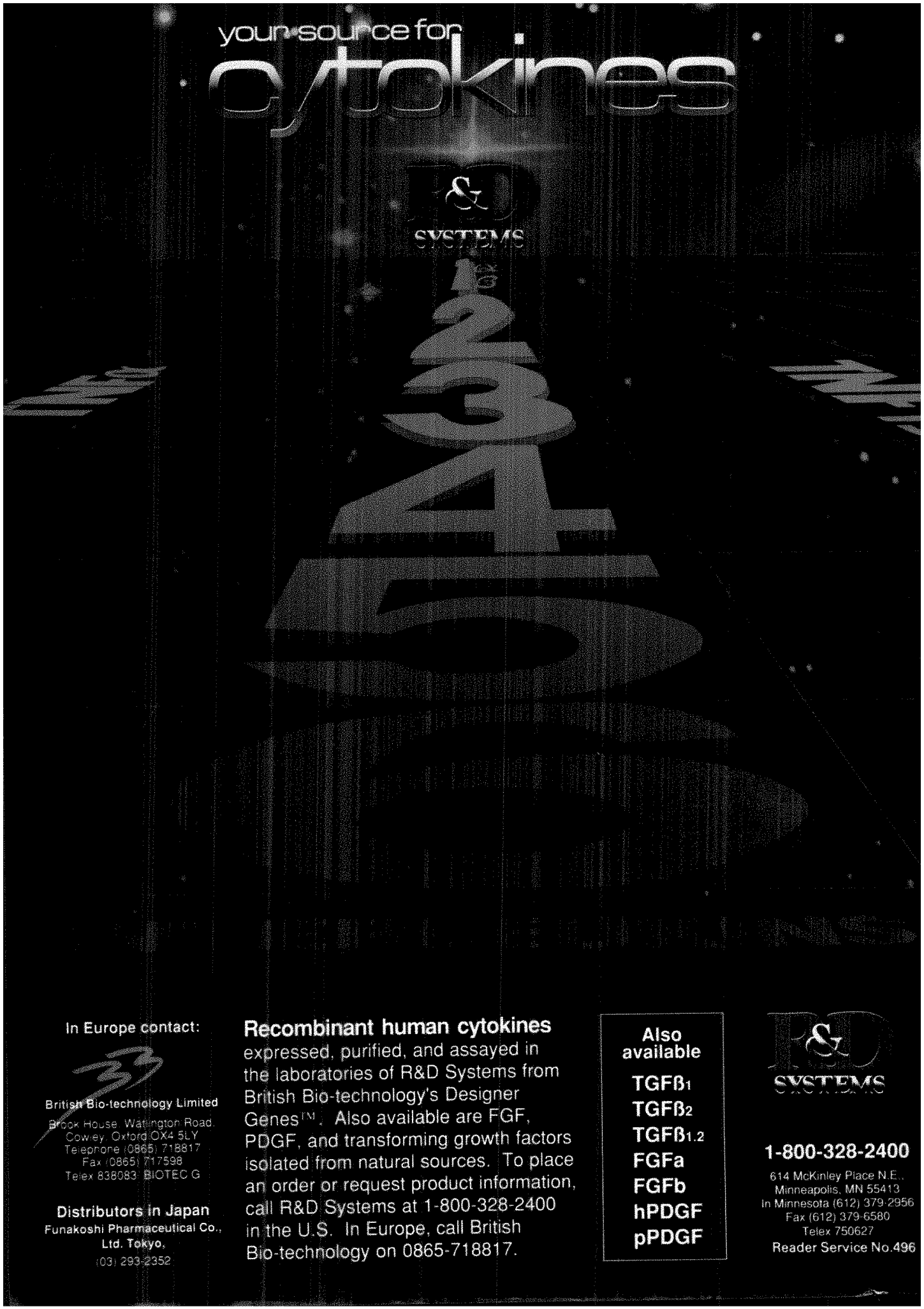
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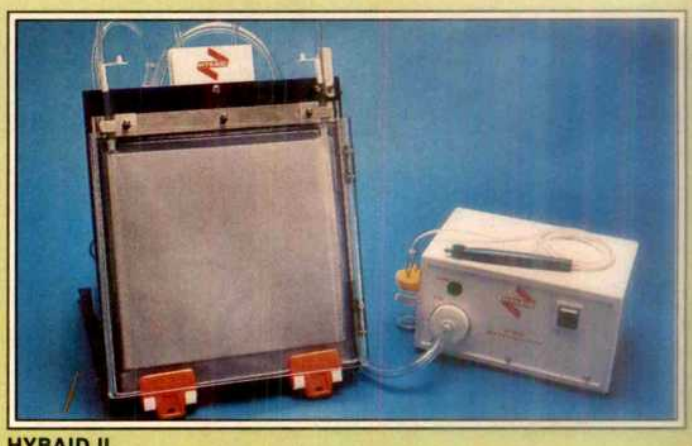
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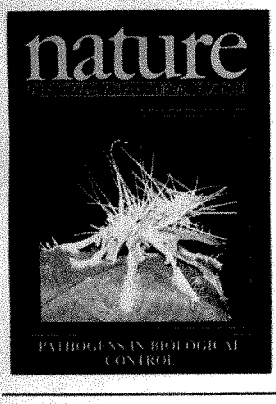
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19 January 1989
Vol. 337 Issue no. 6204

The fungus *Akanthomyces aculeatus* Lebert growing on a leaf-mimicking moth as host, collected at Keravat, Papua New Guinea. Some *Akanthomyces* fungi have sexual states, but *A. aculeatus* relies on wind to disperse its dusty asexual spores. See page 262. Photo: Chris Prior, CAB International Institute of Biological Control.

THIS WEEK

AIDS drug

Soluble CD4, now undergoing clinical trials in patients with AIDS, has a therapeutic effect in an animal model for the disease in which rhesus monkeys are infected with an immunodeficiency virus. Page 267.

Plumbing at depths

What is the relationship between Hawaii's neighbouring volcanoes Mauna Loa and Kilauea? During the past 2,000 years Kilauea has erupted a range of lava compositions resembling historical Kilauea lavas at one end and Mauna Loa lavas at the other — perhaps magma from Mauna Loa invaded Kilauea's 'high-level' magmatic plumbing system. Page 257.

Image maker

These photographs were obtained by projecting an image onto a light-sensitive chemical



system that can act as a novel kind of image processor. Page 244.

Costing deer

The costs of gestation in the red deer, in terms of a reduction in the fitness of the mother, are negligible compared with those of lactation. This observation helps to explain why some mammals, including red deer, commonly produce more progeny than they can afford to rear, page 260.

Graduate quality

The proposition that better teaching is offered by universities also carrying out research is supported by data from the University of Salford. Commentary, page 223.

Improving on nature

Determination of the structure of the ribulose bisphosphate carboxylase active site should facilitate efforts to improve this key enzyme in cash crops by genetic engineering, page 229.

Body heat

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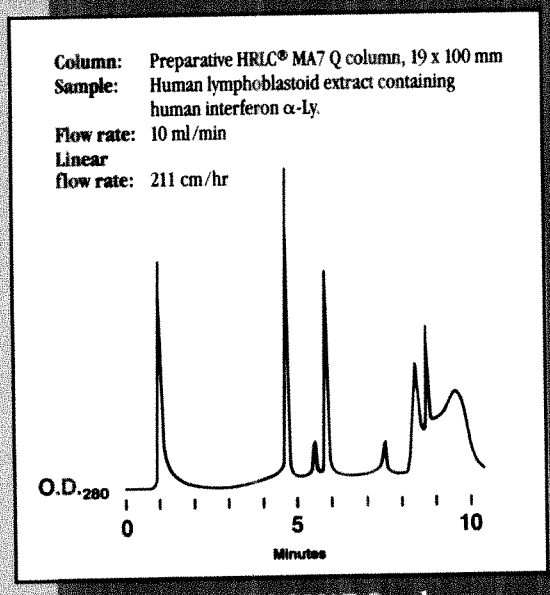
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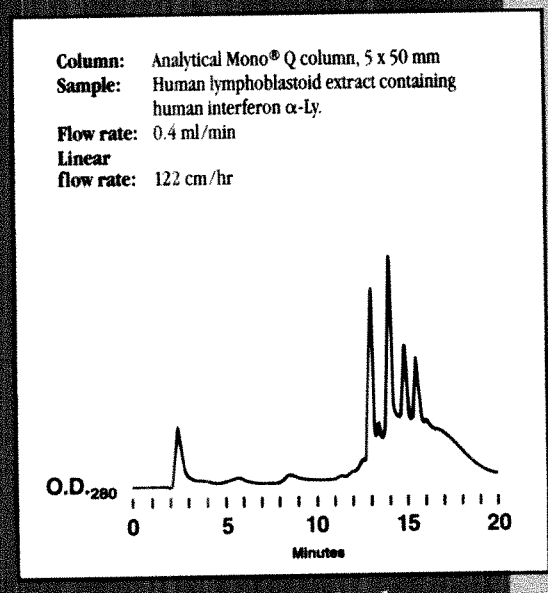
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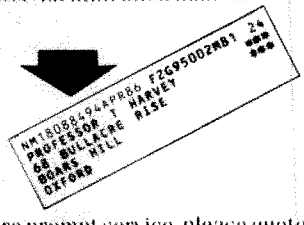
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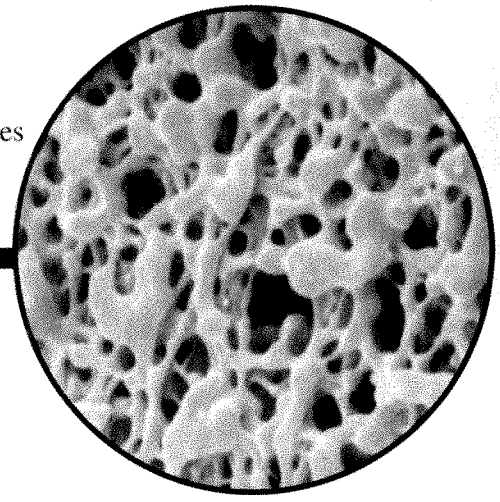
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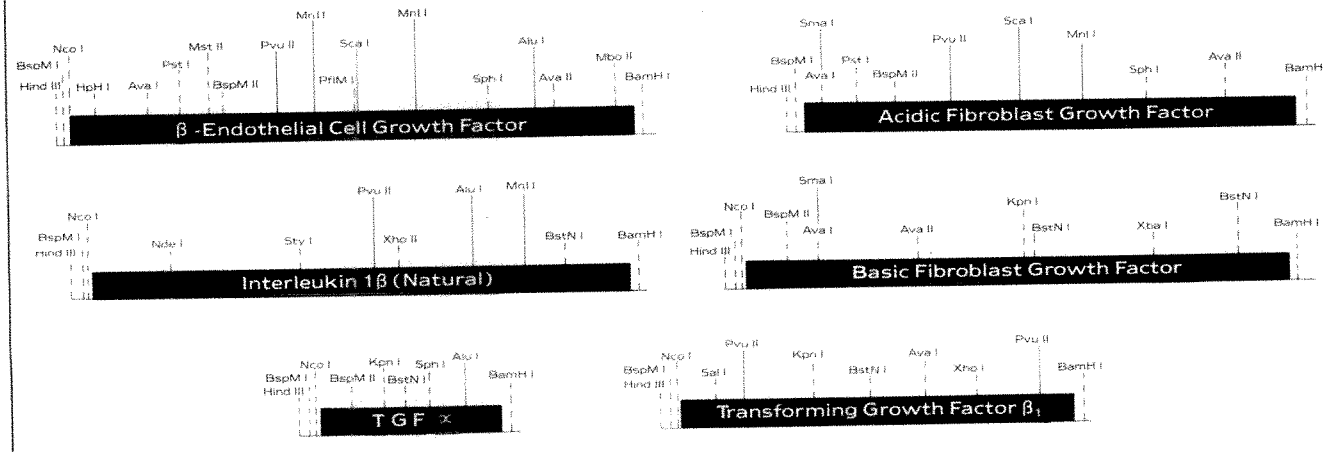
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Ennio Mingolla

Adaptive Hand-Eye Coordination
Michael Kuperstein

Optical Realization of Pattern Recognition
Dana Anderson

Stochastic Neural Net and Optical Learning Machines
Nabit Farhat

Neural Networks for Speech Recognition
Richard Lippmann

Wednesday
Instantiation Schemes for Pattern Recognition
Yi-Tzu C. Chien

Optical Realizations of Bidirectional Associative Memories
B. Keith Jenkins

Mapping Multi-Layered Neural Networks for Pattern Recognition Onto Multi-Processors
Genevieve Cerf

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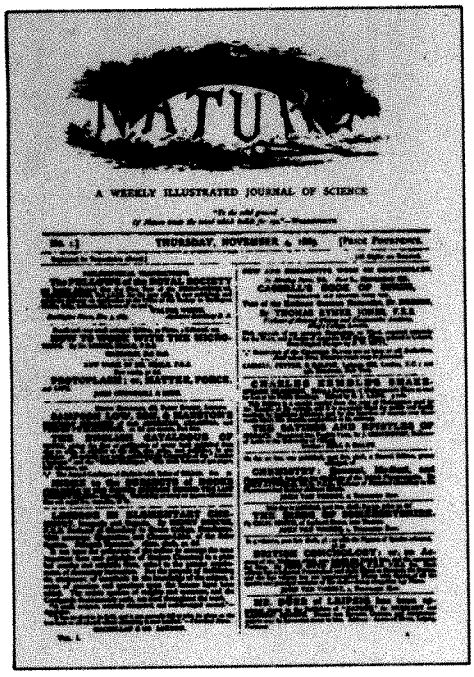
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nature

No gas, please, in the Middle East

Last week's conference on chemical weapons was ruffled by the flawed argument that Israel's adversaries need them to counter Israel's nuclear pretensions.

PUBLIC declarations of good intentions are cheap, decisions to put them into practice much more costly. This familiar truth seems to have been amply confirmed at last week's conference on chemical weapons (see page 199), held in Paris under the now-unfamiliar auspices of UNESCO. The 149 governments at Paris seem readily to have agreed that the best way to avoid the use of chemical weapons is to abolish them, but their joint declaration is deficient in two important ways. First, it fails to say much about the issue that has impeded the negotiations at Geneva for the past eight years, that of the verification of an agreement not to manufacture gases of the kind that can be loaded into munitions, although such a general conference cannot be blamed for that. Second, it fails to specify with clarity the dangers of the precedent set by Iraq during its recent war with Iran, when phosgene or a near relative seems to have been used against troops and civilian populations. Instead, it managed to give general currency to the notion that chemical weapons are a kind of natural counterpoise to nuclear weapons and should be legitimized as such.

That doctrine is profoundly mistaken, but seems to have been made with some force by Middle East states other than Iraq. So long as Israel has nuclear weapons (a plausible but untested assumption), is it not just that its potential adversaries should have access to chemical weapons as a kind of counterpoise? The argument is seductive, but false. The truth is that chemical weapons are not, except in the most symbolic sense, a counterpoise to nuclear weapons. Instead, they are a quite separate nuisance of a most serious kind.

Suppose that Israel is indeed a cryptic nuclear power, with a stock of more than 50 nuclear weapons — so much is the plausible inference from the harsh treatment by the Israeli courts last year of the technician from the Ramona nuclear complex who spilled the beans about plutonium extraction on the site. What use could Israel make of these weapons, which of necessity have not been tested? If Israel were in open conflict with its neighbours, and were so much in danger of defeat that the old Israeli nightmare of being driven into the sea seemed a reality, it is in principle possible that it could save itself from extinction by threatening to use nuclear weapons against the capitals of its adversaries. Earlier use of nuclear weapons, or the threat thereof, would do more harm than good to the Israeli cause — previously friendly governments would be compelled to withdraw support, and worse. But as things are, the possibility that Israel might be extinguished as an independent state would bring friendly governments rallying round long before Israel would rationally need to threaten nuclear retaliation. Thus Israel's nuclear weapons have a military function only in the extreme case that it has lost all its friends and is about to lose a terminal conflict. Militarily, Israel's nuclear weapons are a kind of national suicide pill — but their more immediate political utility is that of helping to cement deals with equally threatened governments.

Chemical weapons are a different kettle of noisome fish. Little is known of the value to Iraq of the use of phosgene in the recent war (somebody should find out), but it is likely to have been marginal in strictly military terms. Soldiers (and civilians) were killed in dreadful ways, but the most substantial damage to the Iranian troops, ill-equipped with protection against gas, is likely to have been psychological. Nerve gases, such as Libya is said by the United States to be preparing to manufacture, would have been more damaging, especially to the civilian population. Their military utility against well-equipped Israeli troops would again be marginal, while their large-scale use against the unprotected population of some Israeli city would bring down on the head of the user an insupportable torrent of protest and constraint. But that does not mean that the prospect that nerve gases will become more widely available is of no concern. These materials would evidently be of great value in surprise attacks against small groups of people, whether troops or not. And, given the ubiquity of the trade in arms, they would undoubtedly be used in other contexts (say, in hi-jacked aircraft) well outside the Middle East.

The argument that chemical weapons are a counterpoise to Israeli nuclear weapons thus makes no sense. That does not mean that the governments in the Middle East that have raised the issue would not be within their rights in demanding that Israel should trade its putative nuclear weapons for the political and military guarantees no doubt accompanying a peace settlement in the Middle East. It may also be prudent to accept last week's demand that the scope of the Geneva negotiations should be broadened to include at least Iraq (although, with 40 participants already, the negotiations are cumbersome enough). But the argument that chemical weapons should be counted as part of a poor country's right to self-defence is paper-thin, and should be denounced as such. To make it is dangerously irresponsible for the curiosity it will excite, not only in the Middle East. □

Industrial illogic

One of Britain's largest companies may disappear because, as in the parable of the talents, it has been over-thrifty.

WHETHER or not 1992 will see the eventual emergence of the true European common market advertised in the 1950s by the Treaty of Rome, the prospect that it might has caused turmoil in what is loosely called the electronics business. Two independent sets of motives have set off a wave of industrial reorganization. European companies, recognizing that there are more of them than the European market can comfortably accommodate, are anxious to consolidate their interests, preferably across European frontiers. Second, companies from outside the European Community are seeking to establish themselves within it, not least as an insurance against the chance that common-market Europe will chauvinistically protect its industries against competitors. The latest reorganization, mostly in Britain, also points to past errors by government and business which are all too likely to be repeated in the future.

The centre of the turmoil in Britain is the General Electric Company, or GEC, which resembles in the pattern of its activity the US company called General Electric or GE, but which is only a third as big. GEC as it is owes its existence to the convic-

tion of the Labour government of 1964 that Britain's four principal manufacturers of power generation equipment then were individually too small to compete internationally and collectively too many to survive; by coaxing and coercion, they were made to merge. The result is a business of extraordinary diversity — it will make you a domestic refrigerator, a computer-aided tomography scanner or a military radar — which has been kept intact and profitable only by the talented administration of its chief executive, Lord Weinstock.

But the conglomerate has failed to make a distinctive mark in any single field; Marconi, technically GEC's pride, conspicuously failed last year to develop an early-warning airborne radar to the British defence ministry's specification (the business went to Boeing instead). Another defect is that GEC first accumulated and has since kept liquid reserves of £1,500 million. But should not companies be thrifty, salting away money for a rainy day? Not if the implication is that they are so dismayed by the difficulties of making money in their own businesses that they ask the banks to try instead. The truth is that GEC has grown too slowly, almost certainly because it has put too little of its own resources into self-financed (as distinct from military) research and development.

Now there is a shake-up on the way. Three years ago, GEC awoke from its cautious lethargy and tried to buy the British telecommunications manufacturer Plessey, but was thwarted by the defence ministry's argument (to the Monopolies and Mergers Commission) that competition among defence contractors would be thereby curtailed. Last year, GEC instead merged its own telecommunications business with Plessey's, but then, this year, cheekily combined with Siemens of West Germany in a second attempt to buy Plessey. That has brought the roof down on its head. Plessey is working hard to recruit support for an attempt to take over GEC, which has been stampeded into merging many of its business with the comparable European businesses of GE of the United States.

The company's misfortunes are partly self-inflicted, partly caused by successive British governments. GEC, like Siemens and GE, began life with the commercialization of electricity a century ago, and became a conglomerate because electricity turned out to have diverse applications. But, for twenty years, GEC has been managed as a collection of separate businesses. That should have given it an advantage over its competitors in being able easily to hive off its activities to others prepared to back their conviction that they could do better with them by paying above the odds for the opportunity. But staying big has been counted more important.

Successive governments have fortified GEC in its complacency by their shortsightedness over defence research. The late Sir Ieuan Maddock, when chief scientist at the then Ministry of Technology, was forever asking why the lion's share of British governments' research and development expenditure should go for aerospace and associated electronics, mostly for the military. The decision to oppose the Plessey merger three years ago was falsely predicated on the belief that the British services must always be equipped by British manufacturers. It would have been wiser if the government had been pressing for a European understanding that the rules of European free trade should also apply to defence procurement. That has now become an urgent need.

Meanwhile, the air is filled with talk of what is called "industrial logic", mostly from people whose skill is the management of money. The calculation in 1964 that the British market could not support four power-generation manufacturers may have been correct, but "industrial logic" would have required that the four should compete with each other for survival; the outcome might have been a manufacturer capable of winning business from elsewhere as well as Britain. In other fields within GEC's present ambit — consumer electronics, for example — there can have been no danger of over-competition a quarter of a century ago. Putting nearly all of Britain's eggs into an oversized company has allowed West German, Italian, Scandinavian and Japanese manufacturers to win handsome prizes. The whole sorry episode should be a reminder to those who talk of industrial logic that success in industry is an empirical, not an abstract, business. The winners are those who succeed in competition with each other. Selling prices matter, but so do quality and performance, all of which are best ensured by intelligent research and development. □

Research in hazard

Last week's US budget promises generosity to science, but only the Congress can decide.

LAST week's valedictory budget from President Ronald Reagan is no less what had been expected of him than the intended tear-jerker of a speech he delivered to the United States a few days afterwards. For the budget, like the speech, has an olympian quality of detachment. Defence spending would increase (but modestly) and, within that, spending on the Strategic Defense Initiative would also be increased. The National Aeronautics and Space Administration would have the funds to build its space station, the Department of Energy would be able to make a start on the Superconducting Super Collider (and the clean-up of its military reactors) and general support for basic science (through the National Science Foundation) would also be increased. So how does the federal deficit affect these plans? On paper, it seems simple. If the cost of social programmes, Medicare and the like, is reduced by $30,000 million, the requirements of Gramm–Rudman can be satisfied for another year. The likelihood that a Congress controlled by Democrats will accept this neat solution may be small, but that will be for President George Bush to worry about when he takes over this Friday.

The danger in this process is that it puts the eminently desirable elements of the outgoing administration's programme in hazard to the uncertainties of a political process that has not yet begun. The next step is for the new president to say how he would amend the Reagan budget. Only then will the Congress begin to say how it will require the package to be changed. It would not be surprising (but par for the course) if the exact balance of the budget for the year beginning on 1 October is not known until after it has begun. In the circumstances, it is natural that the presidents of the US national academies (science, medicine and engineering) should have given the new administration their advice on how to manage science.

The Reagan adminstration's calculation that more spending on basic science would, in the long run, contribute to the competitiveness of US industry and the general wellbeing of society is correct, and in that spirit much has been done to increase, for example, the budget of the National Science Foundation. But some of the more expensive items in what is generally considered to be the science budget, the planned space station for example, are hardly science at all, while there is no known rational basis for telling in advance whether projects of that kind are more likely to yield economic benefits. A stronger science advisory apparatus at the White House could help to open such questions to discussion. Dr Frank Press's argument last year that the time has come when the scientific community in the United States must help to determine priorities is entirely apt.

But will it not be bad for science that one part of the community should be seen to be sceptical of another's pet projects? As in Britain a decade ago, the reality of government support for research and development is determined both by the sums of money set aside in budgets and by the value of what the money will buy when the time comes for spending it. No purpose will be served, in the United States, if promised spending is undermined by rampant inflation or by one of the other consequences of a continuing federal deficit on the scale bequeathed by Reagan to Bush. □

Scandal over Nazi victims' corpses rocks universities

- Television programme opens old wounds
- New mood among medical students

Munich

ISRAEL last week protested to the West German government after a television company broadcast claims that tissue samples and skeletons from the corpses of victims of Nazi-era executions are being used for teaching purposes at West German medical schools.

The Israeli Religion Minister demanded that West German Chancellor Helmut Kohl immediately deliver all body parts from Nazi victims for a proper burial, although it has not been established that Jewish victims are involved. Kohl in turn has called on the universities to stop using any such remains and added that these practices "should have ended forty years ago". The medical schools at Tübingen and Heidelberg are at the centre of the row. Both are looking into the allegations although it is not expected that the charges will be conclusively proved in more than a few cases.

The allegations provoked a storm in West Germany when broadcast on the ARD television network. Although it is well known that, during the Nazi era, corpses for medical education were procured by universities from nearby execution sites, nobody seems to have asked about the origins of the slides and skeletons now being used in anatomy classes.

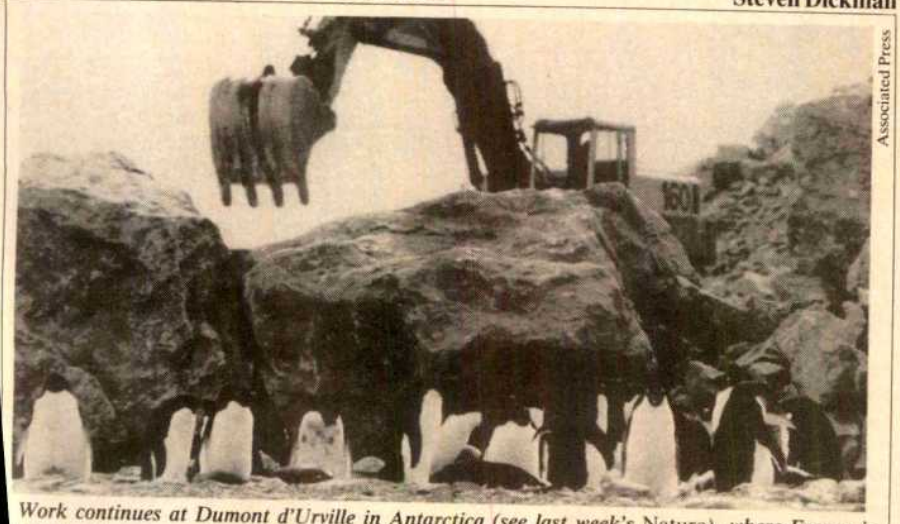
The practice of using the corpses of executed criminals began before the Nazis gained power, but the number of executions rose dramatically after 1933. The anatomy institute at Tübingen alone received 1,077 bodies from the execution site in Stuttgart between 1933 and 1945.

Tübingen University officials said they "had been lulled into a false sense of security" and believed that all remaining body parts from Nazi victims had been disposed of just after the war in a dignified manner. Both Tübingen and Heidelberg promptly began investigations but spokesmen for both universities pointed out that it will be virtually impossible to determine the origins of organs or individual bones in their medical collections.

Officials at Tübingen found four slides that had been prepared from two corpses of Nazi victims who were executed for political reasons. One was a woman of Polish extraction, the other a man presumed to be a German. In Heidelberg, three slides (out of about 1,500 in the collection) were found dating back to 1941 and 1943. The names of the people from whose corpses they were taken were not listed on the slides, but the indication 'decapitatus' indicates that the people may have been victims of Nazi persecution. A skull was also found that had been preserved by the 'preparator' of skeletons employed in the anatomy department from 1937 to 1946. All four items were removed from the department's collection and will be cremated.

That the row has erupted now may be indicative of changing attitudes. The current generation of medical students is demanding a deeper evaluation of the Nazi legacy in West German medicine. One result may be further scandals to come.

Steven Dickman



Associated Press

Work continues at Dumont d'Urville in Antarctica (see last week's Nature*), where France is constructing a new airstrip. Greenpeace protestors are still occupying the southern end of the airstrip.*

Breaking ranks

THE pay dispute between academics and management in Britain's universities took a surprise turn last week when the head of one university made a pay and promotions offer to staff in an attempt to avert industrial action which is disrupting university examinations throughout the country.

The principal of the University of St Andrews, Professor Struther Arnott, offered all academic and related staff a pay increase of 5 per cent from January to March this year and from April to March 1990 conditional on any new national pay scales negotiated. He also offered to make 30 promotions over the next three years, at least 20 to the level of senior lecturer. The offer must be sanctioned by the university's governing body, next month.

The Committee of Vice-Chancellors and Principals (CVCP) was surprised by the move and fears that it may jeopardize the universities' negotiations with the government for extra money to finance salary increases for 1989–90. The Association of University Teachers will not accept the offer, arguing that a local solution will not solve a national problem. But it hopes the move will put pressure on the CVCP to speed up national negotiations. C.McG.

LA cools it

BIOTECHNOLOGY gadfly Jeremy Rifkin has turned his attention to global warming, with the result that before too long there should be pockets of green dotting the asphalt landscape of Los Angeles. Last week, Rifkin joined Los Angeles Mayor Tom Bradley at a press conference at which Bradley announced that the city would plant 2–5 million trees to reduce carbon dioxide emissions and break up "heat islands" that raise the local temperature.

The tree-planting exercise is an initiative of Rifkin's Global Greenhouse Network, a group made up of international public interest groups and legislators. Rifkin expects Los Angeles to be the first of a number of cities around the world to adopt the tree-planting scheme. J.P.

Czech explosives

THE Czechoslovak government, evidently embarrassed by reports that its explosive "Semtex" was responsible for the destruction of the Pan-American aircraft over Scotland last month, last week proposed an international convention on the certification and detection of explosives. The offer, made during last week's conference in Paris (see page 199), immediately preceded the visit to Britain of a Czechoslovak team to assist in the forensic investigation of the disaster. The proposed convention is intended to regulate the manufacture of explosives for legitimate purposes and would include guidelines for their detection and the prevention of their unauthorized disclosure. V.R.

Japan's ERATO programme found to be working well

Tokyo

JAPAN'S Exploratory Research for Advanced Technology (ERATO) programme was set up in 1981 as an experiment to try to break away from the rigid hierarchy of universities and stimulate the kind of creativity that many Japanese researchers think flourishes in the West. Last month, in its first-ever evaluation by a group of foreign researchers, many parts of ERATO pass with flying colours.

The greatest praise was reserved for the Quantum Magneto-Flux Logic Project (1986–91) led by Eichi Goto of Tokyo University, which is exploring the possibility of making ultra-fast computers using magnetic quanta. John Rowell of Bell Communications Research, who assessed the project, said he was amazed that such a "sense of rebellion" could be fostered within the confines of Japan's existing industrial and university structure.

He believes the project has achieved success, as exemplified by the demonstration of a variety of logic functions using single flux elements composed of Josephson junctions. Such progress could not have been made in the United States, he says, where expertise in Josephson junction technology has been allowed to "evaporate".

ERATO, which is run by the Science and Technology Agency's Research and Development Corporation, has financed 18 projects, most of them high-risk research with some potential for application. Two or three new projects are begun each year and funded for five years at $2–3 million per year. Except for the project head, no member of the 15–20 person project teams may be over 35 years old. Researchers are drawn from industry and universities and include several foreigners.

The new evaluation comes from the Japan Technology Evaluation Program, which is supported largely by the US National Science Foundation. The Perfect Crystal Project (1981–86) headed by Junichi Nishizawa, Tohoku University, which developed improved methods for growing perfect crystals of silicon and gallium arsenide, was another project judged "very successful". Edward Wolf, director of the National Nannofabrication Facility at Cornell University, said he was particularly impressed with the effective coupling of the project with industry.

Among the biological projects, the Bioinformation Transfer Project (1983–88) headed by Osamu Hayaishi, Osaka Bioscience Institute, which examined prostaglandins in the central nervous system and their role in biological information transfer, is given high marks by Dale Oxender, director for molecular genetics at the University of Michigan. Seven patents have been filed and the results may have important applications in the pharmaceutical industry, Oxender says.

Seen as successful, but less imaginative, was the Superbugs Project led by Koki Horikoshi of RIKEN, which aims at finding commercial applications for microorganisms that can thrive in extremes of pH, temperature or salinity. The Bioholonics Project (headed by Denichi Mizuno, Teikyo University) and the Molecular Dynamic Assembly Project (H. Hotani, Kyoto University) were seen as "comparable to similar efforts in the United States".

Nearly all evaluators noted that ERATO gives project leaders considerable freedom to spend money as they wish, in contrast to the bureaucratic regulations surrounding financial support from the Ministry of Education, Science and Culture, But university researchers who accept ERATO funds are almost invariably "cutoff" from Ministry of Education funds unless they are "politically powerful". And Rita Colwell, director of Maryland Biotechnology Institute, drew attention to the total lack of female scientists in ERATO and the lack of leadership opportunities for female scientists in Japan.

David Swinbanks

AAU issues guidelines on dealing with scientific fraud

Washington

NEW guidelines intended to help universities to find ways to "distinguish fraud from the honest error and the ambiguities of interpretation that are inherent in the scientific process" were issued last week by the Association of American Universities (AAU), a body that represents most big US research universities.

The guidelines come as public concern over scientific misconduct is increasing, carrying with it the possibility that the US Congress will introduce strict new legislation to control research. A key part of the thinking behind the guidelines is that "institutions ought to be held responsible for the conduct of their faculty", according to Robert Rosenzweig, AAU president.



The new guidelines are simpler and stricter than draft guidelines issued by the Public Health Service (PHS) in 1986 and by the National Science Foundation in 1987. The guidelines do not accept that the resignation of a faculty member can close the books on a misconduct investigation, even though that action may provide an institution's simplest way to avoid public scandal. And once a conclusion has been reached, the institution should discharge its responsibilities to "the public, the sponsors of research, the scientific literature, and the scientific community".

The guidelines take up two of the commonest criticisms of misconduct investigations: that they take too long and that they can cause greater harm to the complainant than to the guilty.

A preliminary inquiry should be completed within 30 days and a full investigation, if warranted, within 120 days, although an institution may "acknowledge formally" that the nature of the case makes it impossible to meet the deadline. The guidelines acknowledge that "younger, less senior people are particularly vulnerable" when making accusations of misconduct, and urges that complainants be protected.

Little is made of another common criticism, that internal investigatory committees are inevitably under pressure to save face for the institution involved.

The new guidelines have been sent to all of AAU's 54 member institutions who will decide for themselves if they wish to use them as the basis for dealing with research fraud. AAU member institutions between them receive 60 per cent of all research grants given out by the National Institutes of Health (NIH), but the PHS, which is responsible for NIH, has yet to complete its own guidelines. An official notice of "proposed rule making" was published four months ago, more than two years after initial guidelines were produced. The final regulations are unlikely to be ready before May.

Alun Anderson

Erratum

Contrary to a News item in *Nature* **337**, 7; 1989 it is Johann Deisenhofer who is now in Texas (at the Howard Hughes Institute, University of Texas Southwestern Medical Center, Dallas) and Hartmut Michel who has remained at the Max Planck Institute (in Frankfurt).

OECD report itemizes risks of renewable energy production

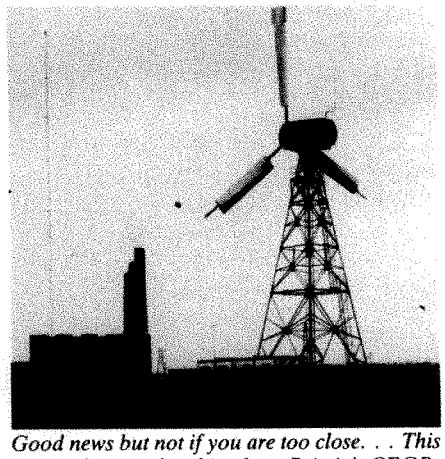
Paris

ENVIRONMENTALISTS have been campaigning for years to alert governments to the long-term risks of burning fossil fuels for energy production, but the so-called 'clean' alternatives of wind, wave, solar power and geothermal power are not without serious hazards, according to a report from the Organisation for Economic Cooperation and Development (OECD).

The report takes the "important positive aspects" of renewable energy use as given. But while the negative impacts of fossil fuels or nuclear energy may be long-term and planet-wide, those of renewables tend to be more localized and shorter-term. In most cases, they arise because renewable sources convert low energy densities and thus require huge collector areas. Hydroelectric power, which accounts for 21 per cent of energy production in OECD countries (1978 figures), like wind power, is not directly polluting, but may render large areas unusable for agriculture or recreation and can destroy local ecosystems.

Biomass, especially firewood, which accounts for about 20 per cent of energy produced in developing countries can, however, have serious environmental impacts. Apart from deforestation, with its risks of soil erosion, burning firewood can produce airborne, inhalable particles that may be carcinogenic, as well as greenhouse gases. Large-scale waste incineration, on the other hand, is only as clean as the material burned. If the waste stream contains plastics or chlorinated products, highly toxic and often halogenated compounds may be produced, including dioxin and hydrochloric acid.

If passive solar energy presents few risks, apart from the build-up of radon due to improved insulation, active solar energy panels contain potentially dangerous chemicals, such as biocides, rust-inhibitors and antifreeze that could pose a problem for disposal on a large scale, says the report.



Good news but not if you are too close. . . This was the first wind-turbine from Britain's CEGB.

Geothermal energy also poses risks. Hydrogen sulphide and benzene are almost always associated with high-temperature geothermal fluids in large volumes and there is also an unknown risk that reinjected geothermal fluids could induce seismic activity — and most geothermal sites are in sesimically active regions.

Peter Coles

Environmental Impacts of Renewable Energy. The OECD Compass Project. OECD 1988.

Reactor restarts *sans* repair

Paris

THE French prototype fast-breeder nuclear reactor, Superphénix, is to be restarted after a 20-month shut-down, even though its leaking fuel-rod transit chamber has not yet been modified (see *Nature* **332**, 384; 1988). The news, published in the government archive *Journal Officiel* last week, follows an announcement by the Industry Minister, Roger Fauroux.

The decision was greeted with applause by the 200 employees at the reactor's Creys-Malville site, near Lyons. But ecology action groups in France and neighbouring Switzerland are outraged and will now seek legal help to try to shut down the reactor permanently.

Superphénix was closed in March 1987 when liquid sodium coolant was found to be leaking from a storage and transit chamber. Engineers from Electricité de France (EDF), the nationalized utilities company that runs Superphénix, found fissures in the inner lining of the chamber. They proposed replacing the sodium coolant with inert argon gas and then running the reactor without the transit chamber inner lining in place. As the core is already charged with fuel, says EDF, the reactor can be run safely for about a year without needing to use the transit chamber. In the meantime, the chamber can be modified.

Fauroux was planning to restart the reactor on 14 January. If there are no incidents, electricity generation could begin again in March.

Critics feel that it is irresponsible to restart the reactor with one of its original safety devices out of action. Should an accident occur within the core, the transit chamber is the only place where fuel rods can safely be stored. They argue that Superphénix is not actually needed for electricity generation and is essentially a full-scale simulator for engineers.

Environmentalists say the government's action is undemocratic. "The decision was designed to have a psychological impact, to demoralize the opposition", says René Longet, director of the Swiss Association for the Protection of the Environment. "Why cannot the French allow open debate on nuclear energy, like other European countries?", he asks. France appears increasingly isolated now that both Britain and West Germany have effectively abandoned their fast-breeder programmes. But France, too, is under internal pressure, mostly economic, and has shelved plans to build a sister to Superphénix. The Centre à l'Energie Atomique, however, remains adamant that it is essential to gain technical expertise at Creys-Malville.

Peter Coles

Indo–French agreement stresses vaccines

New Delhi

A MAJOR Indo–French agreement on medical research is due to be signed in February on the eve of the visit of French President François Mitterrand to New Delhi to open the year-long festival of France in India. The agreement will be between the Indian Council of Medical Research and the French Health and Medical Research Institute (INSERM).

Seven research areas will be covered: fertility and human reproduction, ophthalmology, nutrition, public health, epidemiology, virology, immunology and bioengineering. Collaboration will be on equal terms and will include exchange of scientists, sharing of information and the initiation of research projects. The agreement will continue the trend towards increased collaboration between India and France.

An Indo–French Centre for Advanced Research set up in New Delhi in 1987 has already given shape to 14 joint projects. It will soon have a building of its own, constructed by France and given to India as part of the festival.

Another collaborative venture for the manufacture of vaccines is taking shape. It involves the construction of a plant to mass produce vaccines against measles, polio and rabies, and a multiple vaccine, in Gurgaon, on the outskirts of New Delhi, by the Institut Merieux of Lyons. The plant will at first meet the demands in India and later be expanded to cater to neighbouring countries.

Collaboration on vaccine production is important in the context of India's mission to immunize all children against six diseases by 1992. Another plant for producing 100 million doses of oral polio vaccine is being set up at Bulandshar, 140 kilometres from Delhi, in collaboration with the Soviet Union.

K.S. Jayaraman

Fuzzy computing felt to be the next step in Tokyo

Tokyo

JAPAN's Ministry of International Trade and Industry (MITI) has persuaded a vast consortium of Japanese companies to invest millions of dollars into establishing a new centre for research and development on fuzzy computing.

The decision to set up the facility, called the Laboratory for International Fuzzy Engineering Research (LIFE), was made at a meeting of MITI officials and industry representatives on 11 January. According to a report in the Nihon Keizai Shimbun (an economic newspaper), 42 companies — including Toshiba, Nippon Steel, Hitachi, and Toyota — will each contribute Y100 million ($800,000) to LIFE. The project, not run directly by MITI, will be chaired in alternating two-year shifts, beginning with Hitachi. The centre, scheduled to open in April in Kannai near Yokohama and expected to operate for six years, will be staffed by some 30 researchers drawn from industry and will conduct joint research with government national laboratories (for example, Tsukuba's Electrotechnical Laboratory) and universities (specifically, Tokyo Institute of Technology and Kyushu Institute of Technology). MITI also plans to collaborate with foreign universities and research asssociations in countries such as France, the United States and West Germany. Patent rights will be held by LIFE and licensed to non-members, but fuzzy algorithms will be made freely available.

Fuzzy computing is based on fuzzy set theory, first presented in 1965 by Professor L.A. Zadeh, a US mathematician at the University of California at Berkeley who is now chairman of the International Fuzzy Systems Association there. Whereas conventional digital computers operate on the binary system, fuzzy computers can form conclusions from imprecise data (such as 'faster' or 'not so hot'), treating such input as having a value somewhere in the continuum between zero and one. Proponents of fuzzy theory hold that these characterisitics will allow fuzzy computers to process information in a manner analogous to the brain.

Considerable research on fuzzy computing has already been carried out in Japan, and fuzzy programming on conventional computers is used to control train speed in a subway in Sendai, a city north of Tokyo. At Kumamoto University, Associate Professor Takeshi Yamakawa has developed a fuzzy microcomputer chip set in collaboration with Omron Tateisi Electronics Company. Of the two chips, the 'rule chip' draws inferences from indistinct data; the 'defuzzifier chip' converts inferred conclusions into analog numerical values. In September 1987, the US National Aeronautics and Space Administration (NASA) asked Yamakawa if it could use his technology in controlling attitude and docking in the space shuttle. Yamakawa is said to have agreed on condition that the chips be used solely in civilian applications. NASA is said to have complied. Sometime this spring — after NASA completes its evaluation — Yamakawa intends to commercialize the device.

"What we are interested in falls into three categories", says MITI spokesman Taizo Nishikawa, deputy director of the Electronics Policy Division at the Machinery and Information Industries Bureau, "fuzzy control, such as the Sendai system; fuzzy information processing; and fabrication of fuzzy hardware — for example, fuzzy chips — that will be used to evaluate our basic research in the first two categories."

"There is a very broad range of applications for fuzzy computing", continues Nishikawa. "Industrial applications include image processing, plant management, and robotic control. Social applications include medical diagnosis, weather forecasting and aeronautics control."

The scope of the industries from which member companies come is quite broad, as are their specific interests in fuzzy applications. In addition to the steel, electronics and automotive industries represented by the companies listed earlier, members include computer software houses, chemical companies, electrical appliance and consumer electronics manufacturers, transportation companies, an electric power utility, and — very unusual for a MITI technology project — a Tokyo securities firm.

Stuart M. Dambrot & David Swinbanks

This year's prize season gets under way

London

ROBERTO Poljak of the Pasteur Institute in Paris, Walter Schaffner of the University of Zurich and Gregory Winter of the Medical Research Council's Laboratory of Molecular Biology in Cambridge are to share the 1989 Louis Jeantet Prize in Medicine, awarded for outstanding biomedical research in Western Europe. All three are selected for their contributions to immunology, and each will receive about one-third of the £0.75 million prize, plus £30,000 pocket money.

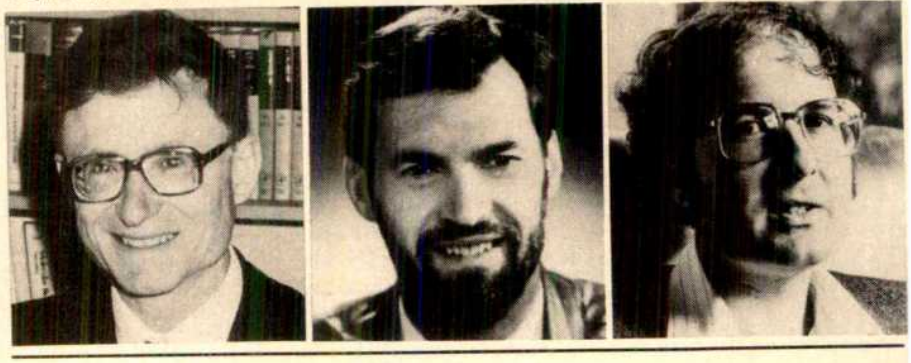
Poljak is cited for his contributions to solving the three-dimensional structure of antibodies, and will use his prize money to improve the computing and graphics power available to his group, and for new area detectors for his X-ray diffraction equipment. He hopes to solve the structure of a lysozyme idiotope–anti-idiotope complex. Schaffner will invest in an automated cell-microinjection apparatus and a fluorescence-activated cell sorter, both to enable his group to study the relevance of DNA methylation to the expression of genes, particularly the antibody genes for which he is cited.

Winter is rewarded for work on engineering antibody molecules, and hopes to finance a project he has been hoping to undertake for some years — to design an antibody molecule to bind specifically to an antigen of known three-dimensional structure. Winter has also won the 1990 Emil von Behring prize given by the Philipps University of Marburg. He wins the £6,000 prize for his contributions to the molecular biology of the development of human monoclonal antibodies.

The 1989 King Faisal International Prizes, each worth £0.5 million, have also just been announced. The medicine prize is shared by Robert Edwards of the University of Cambridge and Luigi Mastroianni of the University of Pennsylvania School of Medicine for work that has made human *in vitro* fertilization possible. Theodor Hänsch, who has recently moved from Stanford University to Munich University, and Ahmed Zewail of California Institute of Technology, share the science prize (this year physics) for their laser studies.

Peter Newmark

Poljak (left), Schaffner and Winter: the immune system pays handsome dividends.



Chemical arms exports embarrass Bonn

Bonn

On 11 January, as West Germany and 148 other nations resolved at a Paris conference to eliminate chemical arms worldwide, the first hard evidence began to appear linking West German companies to the alleged Libyan chemical weapons factory at Rabta. By now, as many as 30 firms have been implicated, although the West German government still insists that it has not seen evidence "admissible in a court of law." One day earlier, West Germany had announced new measures to restrict exports of "sensitive" goods of a military, nuclear or chemical nature. The restrictions came in response to a December scandal in which at least two West German firms are suspected of having delivered nuclear technology to Pakistan, India and South Africa in possible violation of the nuclear non-proliferation treaty.

The revelations about West German participation in the Rabta plant embarrassed the Bonn government. West Germany had been under pressure from the United States to investigate exports to Libya by Imhausen-Chemie GmbH, a company based in the Black Forest. A first investigation by tax authorities in Freiburg in early January cleared the firm.

Chancellor Helmut Kohl refuted the US charge, saying on 10 January that the United States had "put the Germans in the dock without giving us a chance to see the evidence." The next day, the news magazine *Stern* broke the news that the West German customs service had evidence against Imhausen and other firms. At the end of the week, the government revealed that the Federal Intelligence Service (*Bundesnachrichtendienst*) had informed Kohl in October that Imhausen might have violated export laws. The public prosecutor's office in Offenburg has begun a new investigation.

Regulating exports has always been a sensitive matter for West Germany, whose annual exports are worth more than DM 500,000 million. The new regulations put tighter controls on the export of technology and know-how in the nuclear, chemical and munitions areas. The government also recommends increasing the penalties for breaking export laws. The maximum prison term will be raised from three years to five years and the maximum fine from DM 500,000 to DM 1 million.

The government hopes to restrict future arrangements like the alleged Imhausen-Libya deal by lengthening the list of "sensitive areas," which previously included only the Soviet bloc and countries at war. Furthermore, the government recommends prosecuting Germans overseas found to have a hand in the production of chemical or biological weapons.

The opposition criticized the government measures while calling for a parliamentary investigation of the "poison gas affair" as it is coming to be called. Bundestag (parliament) member Norbert Wieczorek (Social Democrat) said it was hypocritical of the government to oppose exports which could lead to chemical arms production only when they go outside the European Community (EC). Wieczorek said that "there are not enough teeth" in the new regulations. He said that the fines discussed were "ridiculous sums" which would be considered by violators as just another operating cost.

Wieczorek admits that tougher controls do not guarantee that undesirable exports will stop. The volume of exports is so great, and the customs and regulatory authorities so understaffed, that many products will continue to pass through undetected. Only by increasing the penalties and the number of spotchecks will would-be violators be discouraged.

Perhaps the thorniest part of the problem comes in the area of "dual-use" technologies, such as the pesticide factory built at Samarra, Iraq, with the help of West German companies. It is has now been established that the factories produced poison gas, which Iraq apparently used during its 8-year war with Iran and on its own Kurdish population. But the guilt of the West German firms remains in doubt because it is unclear if they knew how their products would be used. An investigation into the matter is in its fifth year.

Another firm, Karl Kolb AG of Dreieich, has avoided prosecution under the current export laws by challenging the laws in court. Kolb was one of the companies charged with delivering equipment for the pesticide factory to Iraq. Kolb won the first court case; the government is now appealing.

Steven Dickman

Some good news some bad from Paris

Paris

The five-day Paris conference on chemical weapons closed on 11 January with all 149 participating nations declaring their readiness to "prevent all recourse to chemical arms, by eliminating them completely". But the declaration has already been criticized as not going far enough.

Britain, for example, would have liked concrete measures to prevent the proliferation of chemical weapons and an international commitment to punish governments that used them.

The declaration should, however, speed progress at the Geneva disarmament conference, whose 40-nation membership is trying to formulate a legally binding decree to ban the production and stockpiling of chemical weapons. The declaration asks the Geneva conference to redouble its efforts "urgently to resolve remaining problems" and to draw up a convention.

The plenary committee set up to draft the declaration had to compromise after heated exchanges among the participants. Iran, in particular, wanted Iraq to be pilloried. Syria defended possession of chemical arms so long as Israel has the nuclear bomb. Initial insistence by some other developing nations that there could be no chemical disarmament without nuclear disarmament was eventually dropped. In the final document, individual nations were not named, although the use of chemical weapons in recent wars is condemned.

There was further trouble when the South African foreign minister, Pik Botha, took the platform on 9 January. Many African delegates walked out, as well as one French delegate. But the biggest news from the conference remains the announcement that the Soviet Union will halt production of chemical weapons immediately and will start the ten-year process of destroying its stocks. The Soviet move includes an acceptance that international inspection will be essential to make compliance believable. But the view of the outgoing US administration is that the problems surrounding inspection are "insurmountable", given the ease with which conventional factories can be modified to produce chemical weapons.



Associated Press

Lone voice: Pik Botha spoke but few were there to listen.

Despite the obstacles to a complete treaty, the Paris declaration holds promise as a deterrent to further proliferation. The high profile surrounding the Paris meeting has already helped put pressure on nations such as West Germany, accused of supplying technology to Libya for chemical arms manufacture, to re-examine export controls (see above).

Peter Coles

More money for science in Reagan's last budget

Washington

PRESIDENT Ronald Reagan delivered his final budget to Congress last week, a spending plan that seeks increased support for many areas of basic scientific research. Although it is tempting to view the lame-duck budget as 'dead-on-arrival' — because Congress and the Bush administration are certain to make changes — a complete overhaul of the $1,150,000 million budget is virtually unthinkable, and many of the proposals contained in it are certain to be approved once the new fiscal year begins on 1 October.

The Reagan years have seen substantial increases in spending for research and development. In a speech last week introducing the proposed 1990 budget, White House Science Advisor William Graham pointed out with some pride that between 1982 and 1990 "federal support for basic research will have increased by 56 per cent in real terms", reaching $11,200 million in 1990.

Graham used his budget remarks to criticize groups such as the National Academy of Sciences which have called for a stronger science adviser in the White House. He argued that "creating a science and technology 'tsardom' to direct what is to be done from the top down would be a disaster". Graham believes the best guidance for science comes from individuals doing research, and that a government's role is to support the best research.

The new budget calls for substantially greater spending on several big science projects; the National Aeronautics and Space Administration (NASA) budget requests $2,100 million for the Space Station, the Department of Energy (DoE) will request $250 million for continued development work on the Superconducting Super Collider (SSC), and the National Institutes of Health will be seeking $100 million for the human genome project.

Although the space agency's budget request is considerably more than that for the previous year, NASA administrator James Fletcher says the budget is a lean one, with no room for alteration. He does not hide the fact that the space agency had sought considerably more money, but had its request scaled down by the White House Office of Management and Budget (OMB). Although OMB approved a request for new funds for CRAF, the Comet Rendezvous/Asteroid Fly-by mission and its sister spacecraft Cassini, a planetary mission to Saturn, many prized projects have fallen by the wayside or been cut severely. There is no money for development of shuttle C, a cargo-carrying version of the space shuttle, and the Pathfinder advanced technology programme has been cut by more than half. In addition, OMB has decreed that $208 million must be raised from the private sector to cover expected costs, an ambitious target with which the agency is not happy.

The requested rise of $927 million for defence activities is an indication of the Energy Department's current preoccupation with getting the nuclear weapons programme back on track and bringing its ageing facilities into compliance with environmental regulations. Environmental safety and health programmes will also request significantly more money — $124.6 million in 1990 — but that is only a tiny fraction of the total cleanup cost at weapons facilities, estimated to be at least $50,000 million.

In addition to requesting support for the SSC, DoE's budget also seeks construction funds for a 6–7 GeV Synchrotron Light Source at the Argonne National Laboratory in Illinois and the Compact Ignition Tokamak at Princeton, New Jersey. There is also a request for $27.6 million for work on the human genome project, a $10 million increase over that for 1989.

The National Science Foundation budget seeks $2,150 million in 1990, a 14 per cent increase. Science education continues to receive emphasis, with a total of $190 million for precollege, undergraduate and graduate science education.

The National Institutes of Health (NIH) budget is scheduled for an additional 6.6 per cent for basic research. The total budget for NIH is set at $6,777 million, a modest 3.6 per cent rise over the current figure. This does not include a large increase in spending on research on AIDS, now listed as a separate item in the budget. The total request for federal AIDS activities within the Public Health Service is $1,600 million.

Defence research, still accounting for nearly two-thirds of all federal spending on research, rises to $41,024 million for the Department of Defense. The budget for the National Aerospace Plane is nearly $300 million. The Strategic Defense Initiative request is for $5,800 million, a $1,800 million increase over 1989. The Defense Advanced Research Projects Agency budget request is cut by nearly $166 million to $1,121 million.

Congressional budget committees will begin work in earnest on the budget once they have a clearer idea from President-elect Bush of what changes they can expect. But Albert Teich, one of the authors of a recent report for the American Association for the Advancement of Science on federal spending on research in the 1980s, says there are not likely to be big changes judging by past experience. Teich points out that in 1981, Democrat Jimmy Carter's final budget was filled with signals that the transition to a new administration would bring significant changes when Republican Reagan took over. Those signals are not present today, suggesting that changes to the Reagan budget proposals will be at the margins rather than the core of the spending plans.

Joseph Palca

UK meat research under threat

London

BRITAIN'S meat research could be severely curtailed next year by the threatened closure of the Bristol branch of the Agriculture and Food Research Council's Institute of Food Research, according to the Institution of Professional Civil Servants (IPCS), the union that represents more than half of the Bristol laboratory's 200 staff. The laboratory would be just the latest casualty of the government's policy to withdraw support from near-market research, for which it says industry should pay. The institute's director, Douglas Georgala, confirms that one of the three branches will close, but says no decision has yet been made as to which.

Many AFRC laboratories are suffering as a result of the reduction in contracts from the Ministry of Agriculture, Fisheries and Food, but the meat research laboratory has been hardest hit: its budget of £3.2 million is being halved over three years. The IPCS says efforts by the staff to become independent from government financing and secure contracts with industry are now being blocked: contracts between researchers and the Meat and Livestock Commission have been reduced from three years to two; contracts with industry and government through the LINK food-processing sciences programme will be restricted; and researchers are being prevented from submitting projects to FLAIR, the European food research programme. All these are signs, members of the Bristol laboratory believe, that their institute has been earmarked for closure.

Georgala says every effort is being made to help researchers secure contracts with outside bodies, but a temporary hold on international activities is necessary because the institute does not want to make commitments it may not be able to fulfill. He says the institute's activities will have to be cut back and is preparing a plan to implement cuts which will be presented to the AFRC in the next few months.

Closure of the Bristol laboratory will signify the end of meat research in Britain says the IPCS, and the effect on the meat products industry, worth £8,000 million per annum, will be disastrous.

Christine McGour

Hubble telescope delays prove a boon for some

Berkeley

ALTHOUGH frustrating, the three-year delay in launching the Hubble Space Telescope has proved a boon for at least one of the mission's scientific teams. During ground testing of the faint object spectrograph (FOS), project manager Larry Randall says "fundamental problems" were found that were causing 10–30 per cent reductions in the light-gathering capability of the blue-side and red-side spectrographs. Tests of the instrument's optical alignment revealed a 'cat's eye' where there should have been an unbroken disk of light.

Randall received permission to open the instrument in late 1986. Inside, he discovered insulation and baffle material hanging in the light beam. And pieces of black tape and a rubber glove were loose within the instrument where they could stray into the light path in a microgravity environment. Randall also found that the instrument did not have the proper optical alignment, a problem he thinks may have been caused by the unfamiliarity of the instrument's contractor — Martin Marietta — with proper alignment techniques. Lou Ripp of Martin Marietta who was involved with the FOS project, said through a spokesman that the company had made a mistake in not involving scientists more actively in the design from the start.

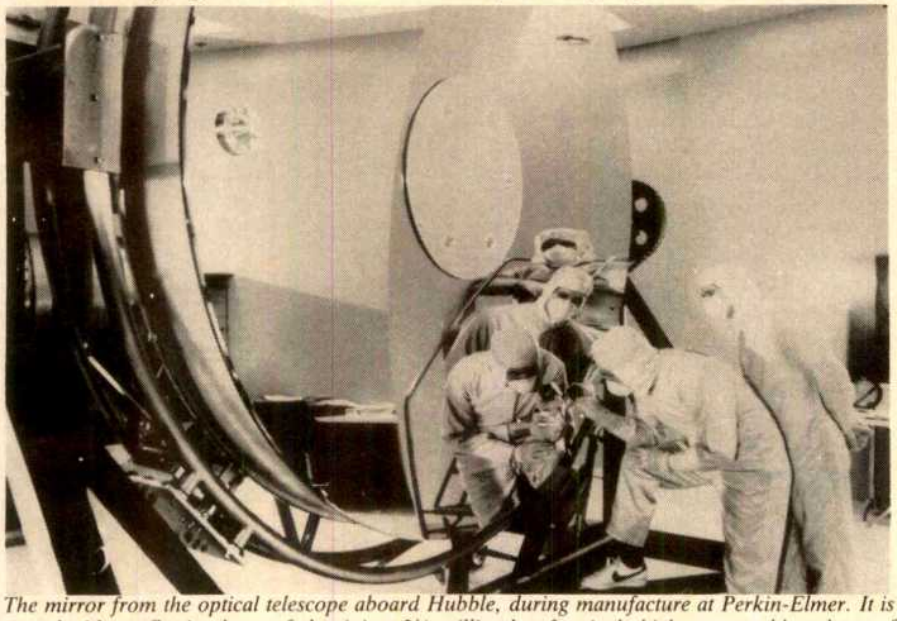
In the overhaul, the FOS light path was restored and the opportunity taken to fit a new detector for the red-side spectrograph. The detector uses recently declassified technology developed by a project member, Edward Beaver of the University of California, San Diego, that has been used in military night vision devices.

The Hubble Space Telescope is now in Sunnyvale, California, where it is in the final stages of ground testing for its December launch. Apart from the changes made to the FOS, the alterations of the telescope's other four instruments were limited to refurbishing of parts to lengthen the lifetime of instruments, and the replacement of a faulty power supply on the high speed photometer, according to Fred Wojtalik, project deputy manager.

The telescope will soon receive its new improved solar wings from the European Space Agency, modified not only to increase power levels by 15 per cent, but also to resist degradation by atomic oxygen encountered in low Earth orbit. The newly developed nickel–hydrogen batteries (*Nature* **326**, 630; 1987) are still in final testing, but if they do not prove suitable Wojtalik says the craft can operate with the batteries from the original design.

The last remaining major decision is whether the telescope will travel to Florida by air or sea. NASA (National Aeronautics and Space Administration) originally planned to ship the craft through the Panama Canal on an ice-breaker. But, because the ship's busy schedule would allow only a narrow time window for the trip, mission planners proposed to fly the telescope to Florida on a C-5A transport, in an Air Force container

The telescope will fly to its Florida launch pad in August unless next month's flight tests show that the Air Force container, which was built for other purposes, is unable adequately to support and protect the telescope, in which case it will go by ship in late spring. **Marcia Barinaga**



The mirror from the optical telescope aboard Hubble, during manufacture at Perkin-Elmer. It is coated with a reflection layer of aluminium 2½ millionths of an inch thick, protected by a layer of magnesium fluoride 1 millionth of an inch thick.

UK plans for astronomy and planetary science

London

A PLAN for Britain's research in ground-based astronomy and planetary science over the next 20 years is published this week by the Science and Engineering Council. It focuses on four projects. The main one is an extension of the MERLIN radio-interferometer, based at the Nuffield Radio Astronomy Laboratories at Jodrell Bank, by the construction of a new 32-metre radio-telescope at Cambridge. This will also enhance British participation in the European Very-Long-Baseline Interferometry Network; construction of the telescope, to begin this year, will cost £6 million.

The council also recommends that Britain seeks a 25 per cent share, at a cost of £2 million, in a new incoherent scatter radar at Spitzbergen in the Arctic and at least a 30 per cent share in a new 1-km gravitational radiation observatory. The council is seeking extra money from the Advisory Board for the Research Councils so that Britain can afford a greater share in the observatory and thus secure its siting in Scotland.

The fourth project in the plan is the construction of an 8-metre telescope optimized for both optical and infrared work. At a cost of £15 million over the next ten years, Britain would hope to have a 50 per cent share in the telescope.

The plan also identifies 17 smaller projects which may be selected according to available resources. £28 million is available for the programme until the year 2000, but the board says that development of the new facilities will require savings in existing ones; these will be made through creating more international partnerships, reducing operating costs and closing some facilities. It does not specify where the cuts might be made. Christine McGourty

Israeli satellite stars

Jerusalem

OFEQ 1, Israel's first satellite, burnt up after re-entering the Earth's atmosphere (over the Pacific Ocean) on 14 January, 118 days after launch. The 156-kg experimental communications satellite was expected to remain in orbit for only six weeks. Professor Yuval Ne'eman, head of the Israeli space agency, attributed the extended life span to the fact that Ofeq 1 (Horizon 1) reached "unexpected" altitudes, thereby encountering fewer particles in space. He said the satellite continued to transmit data on the Earth's environment and magnetic field throughout its orbit.

Ofeq II, a more sophisticated satellite due to carry a scientific payload, is being prepared for launch within two years. With Ofeq 1, Israel became the ninth country to launch a satellite. Lisa Perlman

Anonymous peer refereeing

SIR—Recent complaints about the flaws of anonymous peer refereeing (APR) have overlooked a crucial question: how has this system arisen? Why do authors and referees regard this practice as inherently fair, so that they never really question its ethical validity? The explanation may be an historically interesting example of the unnoticed substitution of values.

The roots of APR are likely to be found in a legitimate democratic perception of anonymous voting. Anonymous voting by, say, secret ballot, is valid when issues must be determined by anonymous majority vote, say the election of governments or departmental chairmen. Scientists as a group have a deep respect for democratic traditions. But judgement on a particular research paper is not within the domain of statistical counting and is not (or at least should not be) subjected to an anonymous peer vote. Is it possible that traditional sympathy for democratic traditions has led the scientific community spectacularly to overlook the inappropriateness of APR?

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SIR—I should like to add my recent experience to the letters regarding anonymous referees from A. Thyagaraja (*Nature* **335**, 391; 1988) and J. B. Wright (*Nature* **336**, 10; 1988). During the past year, I have submitted three reports of theoretical calculations in an active field of research to journals in three different countries. The results of the calculations differed in one significant respect from those generally accepted and publication was refused in all cases. I have no quarrel with this but, as none of the referees was able to produce experimental evidence for the rejection of the results, it was frustrating that all the reports were anonymous.

In one case, I attempted to correspond with the referee through the editor, who refused to pass on my letter as this was againat the policy of the journal. Again, I appreciate that this policy is designed to give referees more freedom to speak their mind, but I would argue that, if referees are to remain anonymous, it should be possible for an author to communicate once through the editor, after which it is up to the referee to consign the communications to the wastepaper basket if he likes.

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SIR—J.B. Wright (*Nature* **336**, 10; 1988) does not believe there is a single good reason for anonymous refereeing, and asks for opinions from other readers. For about twenty years, I have signed all my reviews. My strong feeling is that non-anonymity helps to keep reviews brief, helpful and relevant. Anonymous reviews tend towards rambling essays on epistemology or, worse, a reviewer's opinion, based on unpublished observations, that a particular detail is inappropriate.

Few editors even read unsigned letters. None publishes them. It is therefore inexplicable to me why scientists accept anonymous reviews of their work. Some more specialized journals already publish critiques if they make interesting points not covered in the article itself. *Nature* might try this with some major articles, at least*. The manuscripts would be much improved, and the general reader is likely to enjoy seeing other, informed points of view. Along with Wright, I would have no confidence in a referee who balks at being identified.

There is a further benefit that even the most insecure, but overworked, scientist might appreciate. Judging from a small database, it seems that insisting on having your name on all reviews substantially decreases the number of manuscripts you are asked to review.

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* For an example, see *Nature* **223**, 161 (1969). Editor, *Nature*.

NERC rundown

SIR—The November 1988 issue of *The Biologist* made public what has been known to the Nature Conservancy Council and its advisers for some time, that the UK Natural Environmental Research Council is proposing to run down the Biological Records Centre at Monkswood, a data centre of world renown. The marine science division of NERC has already closed down the long-term biological data series for the English Channel off Plymouth — called the 'Russell Cycle' — and is planning to stop the operations of the Continuous Plankton Recorder survey of the North Sea and eastern North Atlantic early next year. Both of these data series are of world-wide scientific interest, and, like the data held by the Biological Records Centre, provide important information on the effects, deleterious or otherwise, of man's influence on the biosphere.

An inescapable conclusion is that NERC is preparing to close down most of the existing biological recording and collection of time-series data. Such continuing studies have been criticized by research council staff and civil servants on the grounds that they are "open-ended" and prevent the more "flexible" approach required by today's government. In this context, 'flexibility' means the ability of NERC administrators to direct groups of researchers towards special topics currently deemed of popular or governmental importance. Such pop topics change every few years and research would thus proceed in a series of well-publicized short-term *ad hoc* projects. It seems that NERC officials have persuaded their council members to agree that biological recording is a low priority area unworthy of NERC support and that there is a plan in the United Kingdom to run down all biological work of environmental importance, especially in the marine field. It is time biologists started protesting loudly in public and began lobbying fellow biologists who have places on the research councils.

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Folk deceptions

SIR—In his review of Paul Barber's book *Vampires, Burial, and Death: Folklore and Reality*[1], Roy Porter remarks: " . . . this Count Dracula figure, so familiar from the movies, bears scant relation to the traditional revenant who is so powerful a presence in Central and Eastern European folk memory. Examination of recorded sightings shows the vampire was quite different . . .".

As a biologist who is also interested in traditional folk culture, I have spent many years doing field work in the areas that make up Transylvania, yet I have never heard any mention of Dracula, vampires or even "revenants". And in his encyclopaedic survey of ethnohistorical concepts[2], T.A. Szabó Sr includes only a single reference to *drakuly* (meaning 'devil') and that in 1680.

I am inclined to think that the whole Dracula story is a projection of Western European, chiefly Anglo-Saxon, ghost tradition to a distant and mysterious land. That projection has been exploited in print and in movies, best — but not first — by Béla Lugosi, whose picture adorns Porter's review and who was himself a Transylvanian in a broader sense.

ATTILA T. SZABÓ
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1. Porter, R. *Nature* **336**, 283 (1988).
2. Szabó, T.A., Sr *The Historical Thesaurus of Transylvanian-Hungarian Vocabulary* Vols I–IV (Kriterion, Bucharest, 1975–1984).

What prospects for *perestroika*?

The Soviet scientific community seems to have won a substantial measure of freedom in the past year, but the underlying problems of the Soviet economy cast a cloud over change.

Moscow

NOT even the unseasonably warm weather (on at least two days last week, the snow was melting) can explain people's cheerfulness — indeed, most would prefer that the weather would be what it should be at this time of the year, crisp and cold. Much of the explanation is that things have indeed changed a great deal, and for the better. Most noticeably, people who a year ago had never been abroad now seem to be regular travellers to what is still called the West (but which includes Japan). For those not travelling, it is now possible to find periodicals such as the *International Herald Tribune* in the hotels, but shockingly out of date.

There have been notable changes in the regulations affecting scientific work, and in the conscientiousness with which they are carried through, that have simplified once-complicated tasks. On travel, for example, an institute director's decision is usually, now, sufficient.

Similarly, with the publication of research reports, the formal approval of manuscripts by the parent academy (always a formality, but a thief of time) has been abandoned. An institute's director can approve publication in a Soviet journal, but the state copyright board which formally exploits overseas the intellectual property of Soviet citizens must still approve of publication in foreign journals. The decision of at least one writer to defy the board's authority (on the grounds that he would earn more without its intervention) has even raised the question of how long that system can continue to apply to scientific articles (where, usually, no money changes hands).

This betokens a kind of permissiveness that is unfamiliar. While the limits of *glasnost* remain to be explored fully (by experiment) and defined (presumably by reproof of some kind), even those in authority who make no secret of their uneasiness at present trends are evidently diffident at making too much fuss. But Mr Mikhail Gorbachev's 2-hour speech to a gathering of intellectuals at the Kremlin on 7 January, which argued among other things for support and also for self-restraint, may suggest that definition may not be far away.

Meanwhile, people have more elections to occupy their attention. In at least some of the Soviet Academy's institutes, this will be the third occasion in a year in which researchers have been voting on each other's suitability for one or another function. Less than a year ago, institutes were nominating members of the special Party Conference, in October many were electing new directors, now they are nominating people to be deputies in the new constitution's scheme for a representative parliamentary system.

The idea is that the Soviet Union as a whole should elect some 5,000 people to a chamber of deputies that will, this summer, elect from its own number a body known as the Supreme Soviet (at present nominated and largely honorific) to consist of full-time scrutineers of government legislation. The Academy of Sciences has 25 seats in the electoral college (the Soviet Union's philatelists' society also has a quota), the Communist Party as such has 100, but other Party members will no doubt be elected as district representatives.

Academicians Andrei Sakharov and Roald Z. Sagdeev, in that order, seem to be well ahead among the nominations of individual institutes. It is generally believed that, if elected, each would be prepared to stand for the Supreme Soviet. The academy's candidates will be chosen at a meeting this week. There is some ribaldry that one institute, having nominated its own director, decided not to nominate Sakharov as well for fear of undermining its own candidate's chances.

Meanwhile, it is certain that science will be represented at a senior level in the electoral college. Academician G.I. Marchuk, president of the academy, Academicians V. Kotelnikov and Ye. Velikhov (both vice-presidents, the latter now also director of the Kurchatov Institute) together with two other members of the academy appear on the Communist Party's final slate of 100.

Sakharov himself seems gracefully to have made the transition from exile to statesman foreshadowed by his election to the praesidium (governing council) of the academy three months ago. Back from a first visit to the United States only in December, he has been, at the request of Mr Gorbachev, on a fact-finding visit to Nagorno-Karabakh (the Armenian enclave in Azerbaijan) and has caught the attention of the academy with a powerful speech on ecology. If there were still a vacancy as honorific head of state (Mr Gorbachev is now both president and Party general-secretary), Sakharov would surely be appointed instantly.

Not all elections have brought such comfort. There is, for example, the sad case of Academician G. N. Basov, awarded a Nobel Prize in 1964 for the development of lasers (jointly with Charles W. Townes of the United States and his own Moscow colleague A.M. Prochorov) and for many years head of the Lebedev Institute in Moscow.

Basov's star began to set when he was not elected as the institute's nominee to the Party conference. Now he has been succeeded at a reorganized institute (strictly, a federation of research groups) by Academician Leonid Keldysch (son of a previous academy president). The final humiliation is that Basov has not been nominated as a deputy by the national "science-awareness" organization (analogous to the British Association for the Advancement of Science) of which he is the president. Yet he is a vigorous and even kindly man, but lacks adroitness.

Is it possible that, unused to free elections, the Soviet Union equates success in particular elections as a proof of all-round competence (and vice versa)? For that matter, it remains to be demonstrated that the election of institute directors by the staff whom they will manage will always produce satisfactory results.

Elections apart, people are worrying about *perestroika*, the process meant to put the Soviet Union back on its feet. Some welcome the fact that *perestroika* has further emptied the shops of goods on the paradoxical grounds that complacency is the real enemy, but the lack of a clear economic strategy is daunting.

The abrupt change two weeks ago of the rules affecting cooperatives (a kind of euphemism for private businesses) is a more particular worry. Cooperatives making medical diagnostics (small-scale biotechnology companies) will in future be regulated by ministries, others (as in publishing) are forbidden. The origins of this change are obscure, but seem to embody general discontent with the high prices charged by the cooperators, and the consequent high profits.

Meanwhile, too many rubles chase too few goods, so much so that offers to exchange rubles for foreign currency have become almost indecently open. The chief danger for *perestroika* in these circumstances is that of instability. Stopgap foreign credits seem the only quick way out.

John Maddox

Protein structure

Polyhedra for helical proteins

Cyrus Chothia

THE principles that govern how α-helices assemble to form the structure of globular proteins have not previously been understood. But now, in a paper in the *Journal of Molecular Biology*[1], Murzin and Finkelstein present an elegant solution to the problem. Some time ago, these authors showed that the geometries of α-helices packed around a ball-like core can be described by quasi-spherical polyhedra. They went on to argue that the intrinsic properties of α-helices and of their packing surfaces would mean that all or most helical proteins would have a structure described by one of a small number of such polyhedra[2]. In their new work[1], they demonstrate that this is indeed the case.

Three general features of the α-helices found in globular proteins are used in considering ideal helix packings and the polyhedra that describe the geometry of these packings. First, helices have the general shape of cylinders; second, they pack together with one face on the interior of the protein and the other exposed to the solvent; and third, they pack around a central hydrophobic core whose diameter is the length of two residues. Three helices packed in this manner are shown in Fig. 1*a*.

Figure 1*b* shows how the geometry of this packing can be described in terms of a polyhedron. To construct this polyhedron, a sphere of radius 11 Å is first drawn from the centre of the packing. This sphere encloses the central section of each helix axis, and these sections form one set of the ribs of a polyhedron. The polyhedron is completed by another set of ribs formed by the connections linking the ends of the nearest helices (Fig. 1*b*). For the three-helix packing shown in Fig. 1*a*, the polyhedron constructed is the octahedron shown in Fig. 1*b*. Using this construction, the geometry of any packing of helices can be described by a polyhedron.

Murzin and Finkelstein argue that in real proteins the assemblies of helices must be as spherical as possible and that the helices are close packed with a similar number of contacts. The polyhedra whose geometries reflect these properties are those that satisfy four criteria: a convex surface; all faces triangular; all ribs the same length; and a minimal difference in the number of ribs coming to each vertex. Murzin and Finkelstein find that only one polyhedron can satisfy these conditions for a given number of helices if the number does not exceed six. For more than six helices there are no such polyhedra. Ideal three-helix packings are described by an octahedron, four-helix packings by a dodecahedron, five-helix packings by a hexadecahedron and six-helix packings by an icosahedron (see Fig. 1*c*).

Helices can be placed on the ribs of polyhedra in several different ways. Three helices fit on the octahedron in two different arrangements: that seen in Fig. 1*b* and its enantiomorph. Four helices can be placed on the dodecahedron, and five helices on the hexadecahedron, in ten different ways. Six helices can be placed on the icosahedron in eight different ways. (Note in these arrangements the direction of the polypeptide chain is ignored: a helix is just treated as a cylinder.)

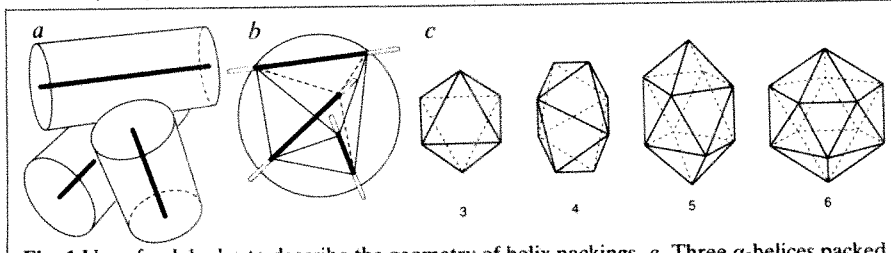


Fig. 1 Use of polyhedra to describe the geometry of helix packings. *a*, Three α-helices packed together are shown as cylinders with a diameter of 10 Å and with their axes as thick lines. *b*, The construction of the polyhedron that describes the geometry of this packing. A sphere is drawn from the centre of the packing with a radius of 11 Å. The parts of the helix axes enclosed within the sphere form one set of ribs of the polyhedron. The other set of ribs is formed by the lines joining the nearest ends of the helix axes. *c*, The quasi-spherical polyhedra for the ideal packing of three, four, five and six helices.

This 'quasi-spherical' polyhedron model describes the ideal packing of simplified approximations of α-helices. To determine if the model is relevant to real proteins, Murzin and Finkelstein compare the geometries of the helix arrangements observed in real protein structures with those expected from ideal models[1]. They make comparisons for all the proteins that contain helix packings and for which atomic coordinates have been published. In almost all cases there is a close fit between the helix packings found in these proteins and one of those in the ideal models. The larger proteins are formed by a combination of simple polyhedral packings. I show in Fig. 2 the three- and five-helix packings observed in actinidin[3] and thermolysin[4] and the corresponding ideal models.

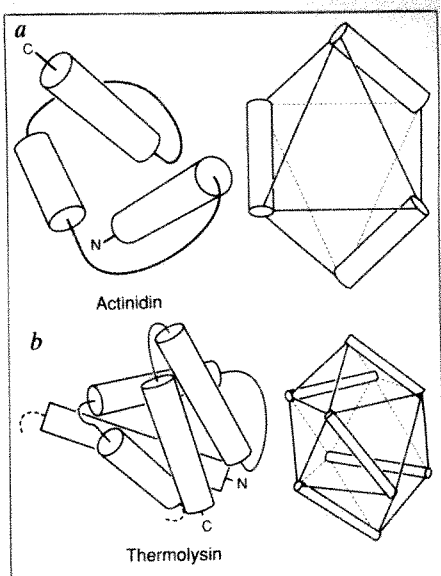


Fig. 2 Observed helix packing and ideal models. *a*, The three-helix packing found in actinidin[3] and *b*, the five-helix packing found in thermolysin[4]. The drawings were made by fitting cylinders to the atomic coordinates of the helices. The ideal helix packings whose geometry corresponds to that observed are shown on the right.

The positions and orientations of helices in the observed structures differ from those found for helices in the corresponding ideal model by 2 Å and 20° on average. The larger deviations occur in proteins where the spherical nature of the packing is distorted by the helices clustering not about a point but about a somewhat more elongated or flattened region.

The absence of ideal polyhedra for assemblies of more than six helices suggests that in the few proteins that contain such assemblies the packing is described by different types of models. One possible alternative would be the formation of layer structures by the helices[5], like those in proteins containing β-sheets[6,7]. The low-resolution structure of bacteriodopsin[8], for example, suggests that it is formed by seven helices packed in two layers.

Analyses of protein structures show that pairs of close-packed helices usually have their axes tilted in one of two directions: at angles of approximately −50° or +20°, arising from the ridge and groove character of helix surfaces[9]. The polyhedron models in which pairs of neighbouring helices are in orientations close to these values are those that most commonly correspond to the observed structures. There are two models for the packing of three helices, for example. That shown in Fig. 2*a* has the axes of the packed helices inclined at −60°, a value close to the preferred orientation of −50°, whereas in the alternative model the angle is +60°, which is some way from the nearest preferred value of +20°. Of the eight

non-homologous three-helix packings observed, seven are described by the first model and one by the second[1].

The quasi-spherical polyhedron model for the packing of helices has important implications for fields outside protein folding. Similarities in the structures of proteins are often taken to imply evolutionary relations even if there is no significant homology in sequence. Such a relation may exist. But Murzin and Finkelstein show that helical proteins can have very similar architectures independent of both sequence homology and chain pathway. Helix packings from non-homologous proteins but whose geometry is described by the same model appear very similar. Indeed, helix packings whose connectivities are very different but which fit the same model have sets of alpha carbon atoms that superpose with a root-mean-square difference in position of less than 2.5 Å. Values somewhat greater than this have previously been taken as clear evidence for evolutionary relationships. □

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Computation

Breaking the matrix speed limit

Steve McCormick

MATRICES abound in the sciences. Many problems involving inter-related variables can be cast into equations that use the familiar array form of a matrix. For large-scale problems involving many variables, the matrix in question is often composed mostly of zeros — a so-called sparse matrix. To capitalize on this structure, the equations can usually be solved by iteration on a computer. Unfortunately, the asymptotic approach of the iteration to the solution can become extremely slow, a process termed critical slowing down. Among the many possibilities for combating this stumbling block, that now used by R. G. Edwards *et al.* (*Phys. Rev. Lett.* **61**, 1333–1335; 1988) in a model problem shows great promise.

Sparse matrices arise in the modelling of large, complex phenomena from virtually every corner of science, including fluid mechanics, structural analysis, molecular dynamics, geodesy, economics, image reconstruction and X-ray crystallography. A sparse matrix expresses known local rules that govern the behaviour of a system of objects describing the phenomenon of interest. These rules characterize direct interactions that emerge as numbers in the matrix array. The numbers in a given row quantify the interaction of a given object with all of its neighbours. The location of the relatively few non-zero numbers in the row indicate who the neighbours are, and their magnitudes indicate the degree of the interaction. The actual behaviour of the system can be recovered numerically by solving a set of equations defined by the matrix, but conventional methods for solving these equations often suffer from the debility of critical slowing down.

In their new work, Edwards *et al.* investigate this sort of trouble in the context of certain lattice systems that arise in the study of random-resistor networks, quantum chromodynamics and discrete Schrödinger operators. (For example, random-resistor networks are a paradigm for the conduction of current through composite materials. The non-zero matrix elements correspond to resistors randomly placed between neighbouring points of a lattice, and the idea is to determine the current at these points for a given external current supplied to the lattice.) The cure Edwards *et al.* use involves a relatively new method, called algebraic multigrid (AMG), the basic idea of which can be described as follows.

Conventional methods start with a given approximation to the state of the system, then attempt to make improvements by a sequence of sweeps that adjust the state of each object in the system, forcing the objects in their turn to obey the local rules. Critical slowing down occurs after a few such sweeps because these local adjustments can take an inordinate amount of time to determine the global state of the system. This is not unlike the trouble that an ant has in building or, better, rebuilding an ant hill. AMG avoids this trouble by making additional improvements on more global scales.

This is accomplished systematically by aggregating the objects into groups, automatically translating the local rules to apply to the aggregated system, then sweeping through these groups to make adjustments at the aggregated level. This approach gives an ant the power to work with clusters of sand for adjusting the coarse features of the hill, reserving the delicate manipulation of individual grains for the finer features. It gives the researcher the ability to determine the state of a system at a cost equivalent to just a couple of sweeps of the conventional method.

This approach is often relatively easy to develop, at least in principle. For lattice systems, there is usually one property (or variable) used to express the local rules among the objects, and this property can be treated as sort of a metric to determine appropriately how the objects and local rules should be aggregated. For example, in random-resistor networks, the resistance between two sites that are geometric neighbours can be interpreted as a measure of the intervening distance. Sites that are close in this sense can be grouped, and the aggregated rules can be determined by an averaging of the individual rules based on this measure. AMG computer software that automatically implements this approach has indeed been fairly successful for lattice systems, elliptic fluid flows and various other applications.

But for other complicated phenomena, development of the AMG approach can require very substantial research. For example, many systems use two or more properties per object to define local rules. In structural analysis, displacements for each coordinate and rotations for each pair of coordinates determine the state of the system. In developing an AMG scheme for a new problem of this type, just how such properties should be used to form distance measures is not always clear at the outset. But there have been very successful designs of AMG techniques for a broadening class of applications, including least-squares problems in geodesy, general fluid flows and various structural-analysis problems (see *SIAM Frontiers in Applied Mathematics* Vol. 3, ed. McCormick, S. F.; SIAM, Pennsylvania, 1987).

But this is just the beginning. Led by its pioneer A. Brandt and others, the multigrid concept has been making dramatic inroads into many new areas of science (see *Lecture Notes in Pure and Applied Mathematics* Vol. 110, ed. McCormick, S. F.; Springer, New York, 1988). Several challenging problems that were previously thought to be intractable now seem ready to fall before the multigrid axe. In the field of quantum chromodynamics, it is estimated that conventional methods could take ten million years or more to determine the mass of a proton. A novel multigrid approach seems to hold the promise of reducing this time to perhaps as little as a few hours. If such dramatic improvements can be made in this and other challenging fields, then critical slowing down may be well on its way out of scientific computation. □

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Gene transcription

Waves of DNA supercoiling

Maxim Frank-Kamenetskii

IF YOU walk to the window and rotate the double string used to draw the blind in a clockwise direction long enough to obtain a double helix, then insert a pen between the two strands and push it ahead without rotation, you would be making a mechanistic model of the transcription process: the pen, or RNA polymerase, moves along DNA, the interwound double string. From this experiment, it can be seen that while RNA polymerase translocates it should overwind DNA in front of itself and underwind DNA behind itself. In other words, DNA becomes positively supercoiled in front of RNA polymerase and negatively supercoiled behind it (see figure). Though an inevitable consequence of the DNA helical structure, this wave of supercoiling which moves with RNA polymerase seems fantastic. However, the elegant recent experiments by J. C. Wang and co-workers demonstrate that these waves of supercoiling actually exist both in prokaryotic and eukaryotic cells (Wu, H.-Y. *et al. Cell* **53**, 433–440; 1988; Giaever, G.N. & Wang, J.C. *Cell* **55**, 849–856; 1988). These data will surely change the general attitude to the biological significance of DNA supercoiling and to the importance of DNA topology.

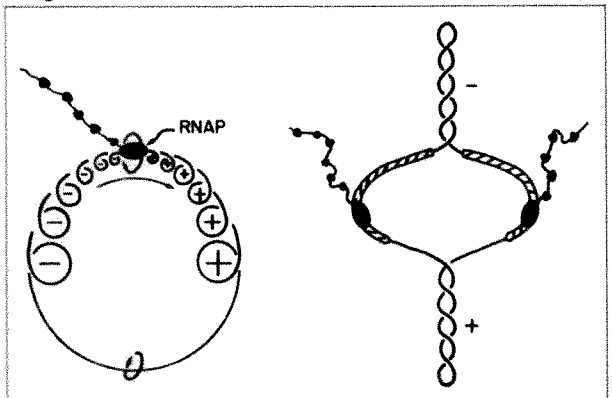
Continuing the experiment with the string and pen, very soon the pen stops because the double string cannot be overwound any more. Thus, one has to suppose either that DNA and RNA polymerase rotate around each other, or that the cell can eliminate both positive and negative supercoils. One can hardly expect that a very long DNA molecule and a very bulky transcription machinery loaded in prokaryotes with an even bulkier translation machinery could rotate around each other. On the other hand, topoisomerases are known to be able to change DNA supercoiling. On the basis of these simple reasons, L. F. Liu and Wang put forward the concept of waves of supercoiling (*Proc. natn. Acad. Sci. U.S.A.* **84**, 7024–7027; 1987). But how can the wave of supercoiling be measured? Extraction of DNA from the cell, ridding it of proteins, causes the memory of the wave to be lost, as the wave of supercoiling does not change the overall DNA linking number. Although Wang and co-workers could not directly observe the wave of supercoiling *in vivo*, they unambiguously demonstrated its existence in experiments involving the inhibition of different DNA topoisomerases. Their most striking piece of evidence is the formation of positively supercoiled plasmid DNA in *Escherichia coli* when DNA gyrase is inhibited. In this case, topoisomerase I continues to remove negative supercoils, while positive supercoils, which are normally removed by DNA gyrase, are accumulated in DNA.

In contrast with the prokaryotic case, one cannot selectively cut off the topoisomerase activities which relax positive and negative supercoils in eukaryotes. Giaever and Wang overcame this difficulty by using yeast temperature-sensitive (*ts*) strains transformed by a plasmid expressing *E. coli* DNA topoisomerase I. This enzyme relaxes negative supercoils but cannot relax positive ones. In full agreement with the model, an endogenous plasmid extracted from cells grown under nonpermissive temperature when yeast endogenous topoisomerase was inactive, turns out to be significantly positively supercoiled.

These findings throw a totally new light on the biological significance of DNA supercoiling. Specifically, people had thought that DNA gyrase in *E. coli* serves as an enzyme which introduces negative supercoils into DNA. Competing with topisomerase I, it supports the native level of DNA supercoiling in the cell. Some held that by changing DNA supercoiling, gene expression could be regulated.

Now it can be seen that this picture, which was almost generally accepted, is really upside down. Indeed, DNA gyrase seems to eliminate positive supercoils rather than to create negative ones in *E. coli*. Native supercoiling is an irrelevant notion because actual local supercoiling may be highly positive, highly negative or negligible depending on the position of promoters, on the current position of RNA polymerase and on the relationship between the rate of RNA polymerase translocation and the efficiency of supercoil removal by the topoisomerases. Supercoiling depends on transcription to a far greater extent than transcription depends on supercoiling.

The findings of Wang and his colleagues emphasize the significance of the unusual structures in DNA (open regions, cruciforms, Z and H forms and so on) which form *in vitro* under negative supercoiling. These structures can form transiently in the negative domain of the wave of supercoiling (behind a moving RNA polymerase) and be trapped by specific proteins. Among other things, this could explain the transcriptional activation of replication and recombination. □



Formation of twin supercoiling domains during transcription. Left, a single RNA polymerase (RNAP) transcribing along a plasmid. Translocation generates a positively supercoiled domain (+) in front of RNAP and a negatively supercoiled domain (−) behind it. Right, when two opposing transcripts are present on the same circular DNA, the two supercoiled domains cannot merge by rotating the DNA alone; one of the transcription ensembles must be rotated as well. (From Wu, H.-Y. *et al. Cell.* 53, 433; 1988.)

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100 years ago

THE BALD-HEADED CHIMPANZEE



THERE is no longer any room for doubt amongst naturalists as to the complete distinctness of the larger anthropoid ape of tropical Africa, the gorilla, from its smaller brother, the chimpanzee. But, on the question whether there is only one chimpanzee over the African continent, there is still much difference of opinion. The acquisition of the fine female specimen, known by the name of "Sally", by the Zoological Society in 1883, caused me to change my views. There can be no doubt that this animal, when compared with specimens of the ordinary chimpanzee, presents very essential points of distinction. The uniform black face and nearly naked forehead, render "Sally" conspicuously different from the many specimens of the common chimpanzee that the Society has previously received.

From *Nature* **39**, 254; 10 January 1889.

Geochronology

Diamond dating anomalies

Martin H. Dodson

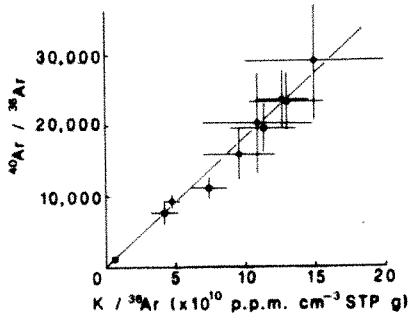
DIAMONDS are particularly interesting samples of the Earth's mantle. Because of their unique combination of chemical stability and mechanical strength, impurities which they incorporate at their birth are protected almost indefinitely against interactions with the surrounding mantle. Some diamonds are known to be 2–3 thousand million years (Gyr) old[1,2], but direct dating of diamonds by the potassium–argon method has not been attempted until fairly recently[3,4], despite the great sensitivity of modern equipment for argon isotope analysis. Expectations, however, in geochronology are easily confounded; Ozima and co-workers, in a series of papers[4–6] culminating in the one on page 226 of this issue[6], show that forming diamonds can incorporate large amounts of 'excess' radiogenic argon in association with potassium, so that what began as a dating project has ended as a study of ancient mantle fluids.

Because of previous indications that excess argon would be a problem[3], Ozima and his colleagues decided to use an 'isochron' method (see figure) to date a suite of ten samples from Zaire called 'cubic' diamonds. These curious stones are polycrystalline aggregates with a rather fibrous structure and roughly cubic in shape, some having clear octahedral cores. It seemed likely that they are of uniform age and share the same initial argon isotope composition, essential conditions for the isochron method. In the event the stones yielded[4] a respectable linear array on the potassium–argon isochron diagram — indicative of uniform age — but a thoroughly unrespectable age of 6 Gyr, apparently making them 1.5 Gyr older than the Solar System.

Obtained from a single sample, this result would simply have been rejected as being due to excess radiogenic argon. The linearity of the isochron plot, however, made such an idea hard to accept, for it required that the excess argon was incorporated in exact proportion to the potassium. This seemed unlikely, because normally the contrast in chemistry between potassium and argon ensures that the Ar/K ratio in a new mineral grain is both lower (commonly zero) and more variable than in its surroundings.

A more fundamental explanation was therefore sought, and it was suggested[4] that the potassium had an anomalously high abundance of the parent ^{40}K. However, it was soon shown[5] that potassium in Zaire diamonds is isotopically indistinguishable from that found in any other terrestrial material. Thus the diamonds must either have incorporated a pre-solar component (diamonds have been found in meteorites) or have been isotopically contaminated in a very unusual way.

The question is now resolved in this issue[6] in favour of contamination during crystal growth, by mechanical entrapment of an argon-rich fluid. The clinching evidence comes from the argon–argon (or argon-39) method, in which samples are irradiated with fast neutrons, converting a small proportion of ^{39}K to ^{39}Ar. The ratio ^{40}Ar/^{39}Ar in gas extracted from the



Isochron plot for cubic diamonds from the earlier paper by Ozima and co-workers[4]. The ^{40}Ar and K contents of samples are plotted as ratios to their ^{36}Ar contents, which are assumed to be constant through time. Because ^{40}K decays radioactively to ^{40}Ar, data from samples formed at the same time, with uniform 'initial' argon-isotope composition (^{40}Ar/^{36}Ar), but *variable* K/^{36}Ar, would all fall on a straight line whose slope gives the age of the samples. The intercept shows the initial ^{40}Ar/^{36}Ar, in this case close to zero (−700 ± 900), suggesting that no ^{40}Ar was trapped in the forming diamonds. The ^{39}Ar data of Ozima and co-workers in this issue, however, show that these diamonds entrapped, as they grew, a variable mixture of fluids, one with high, fairly uniform Ar/K, another with low ^{40}Ar/^{36}Ar.

samples corresponds to the radiological daughter–parent ratio ^{40}Ar/^{40}K in the conventional method. The gas is usually extracted in stages while the sample is heated to successively higher temperatures; changes in apparent age during this step-heating procedure show differences between the locations of ^{40}Ar and ^{39}Ar within the sample. (Furthermore, during neutron irradiation ^{38}Ar is produced from chlorine and ^{37}Ar from calcium, so the concentrations of these elements may also be determined by argon isotope analyses.)

In their new paper, Ozima *et al.*[6] present step-heating analyses of four irradiated diamond samples. The 'age' from their isochron plot is again close to 6 Gyr, with little indication of changes during step-heating. More important, they find an excellent correlation of chlorine with ^{40}Ar. Ozima *et al.* point out that similar correlations obtained from fluid inclusions in the upper crust reflect leaching of argon and chlorine from crustal rocks. Evidently these diamonds provide evidence for analogous processes in the mantle, and the gases extracted from them consist of mixtures, in varying proportions, of a chlorine-free component with ^{40}Ar/^{36}Ar of about 340 and a component with ^{40}Ar/Cl of 8 or 9. Rather less convincing is the attempt, by means of a correlation diagram for radiogenic ^{40}Ar/K against Cl/K, to separate the ^{40}Ar into an excess component and an *in situ* radiogenic component (with a calculated age in the range 0–3 Gyr).

Navon *et al.*[7] (see also ref. 8) recently presented elemental analyses for Zaire cubic diamonds rich in sub-micrometre inclusions, interpreting them in terms of volatile-rich fluids roughly comparable with potassic magmas. Ozima *et al.* give potassium, chlorine and calcium abundances which are broadly consistent with those of Navon *et al.*, and provide complementary argon-isotope data. The significance of these inclusions for the understanding of mantle processes[8] would be hard to exaggerate, and it is clear that there must be further attempts to date them. Reported success in dating of fluid inclusions in crustal rocks[9] suggests that the Rb–Sr method could be worth a try.

Finally, do the problems encountered in dating of diamonds have serious implications for more conventional applications of the potassium–argon method? The answer is, probably not. In these diamonds the potassium–argon system is evidently dominated by a rather homogeneous ^{40}Ar-rich fluid which has been incorporated mechanically during crystal growth, mixed with small amounts of a low ^{40}Ar/^{36}Ar component. Thus the system does not satisfy one of the most fundamental requirements for successful dating, that the radiometric clock must be reset by a process of chemical differentiation between parent and daughter elements. In ordinary rocks and minerals the immense difference between the chemical properties of potassium and argon ensures that this condition can usually be satisfactorily met, with the likelihood of obvious inconsistencies in the data if it is not. □

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Vertebrate neurobiology

Sound localization mechanisms

David Moore

DIFFERENCES in the intensity and time of arrival of a sound at the two ears are well known to be the primary cues for auditory spatial localization in vertebrates (*a* in the figure). But despite many speculations, the neural mechanisms underlying these processes are not well understood. In two new reports[1,2], Masakazu Konishi and colleagues demonstrate the cellular and topographical neural organization underlying the first stages of processing of these signals in barn owls.

Barn owls are one of a small number of owl species to have evolved bilateral ear asymmetry. Such species are suited for studies of sound-localization mechanisms, because interaural time difference (ITD) contributes exclusively to localization in the horizontal plane (azimuth)[3] and interaural intensity difference (IID) forms the sole basis for vertical plane (elevation) localization[4]. The time and intensity pathways form separate streams in two divisions of the cochlear nucleus[5,6], the first synaptic station in the brain's auditory system. Neurons of the cochlear nucleus receive input from only one ear and so cannot detect ITD and IID. Detection therefore begins at the next level of the auditory system, where ITD processing occurs in the nucleus laminaris and IID processing in the posterior division of the ventral nucleus of the lateral lemniscus.

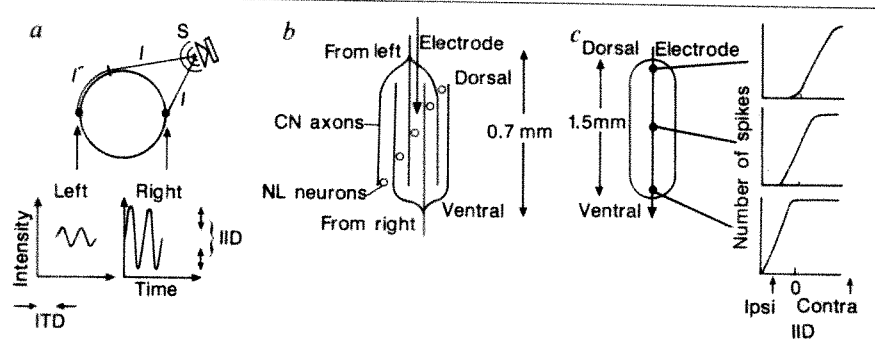
Jeffress proposed 40 years ago that ITD processing would be based on delay lines and coincidence detection[7]. He predicted that the length of axons derived from each ear and synapsing with binaurally innervated neurons should vary to compensate for physical time differences between the ears created by laterally positioned sounds. Thus, for a sound located on the right side of a barn owl's head, signals generated in the right cochlear nucleus would travel through longer axons to arrive at left nucleus laminaris neurons coincidentally with signals that had been generated later in the left cochlear nucleus. This model also predicts that nucleus laminaris neurons should be topographically arranged so that coincidence detection corresponding to different regions of space varies systematically across the extent of the nucleus.

Carr and Konishi now provide[1] direct evidence for both these proposals. By filling single axons from the cochlear nucleus with the tracer horseradish peroxidase, they show that axons derived from the two ears enter the nucleus laminaris from opposite sides and interdigitate (*b* in the figure). Physiological recordings from these axons reveal progressive shifts in the timing (phase) of responses relative to tone stimuli delivered to each ear. Through the depth of the nucleus laminaris (0.7 millimetres), timing shifts for stimulation of either ear are about 200 microseconds, consistent with the behaviourally relevant range of ITDs. But timing shifts for the two ears are in opposite directions, as predicted from the anatomy. Recordings from the dorsal side of the nucleus laminaris show a time lead for the ear on the same side as the recording, whereas ventral recording sites show a time lead for the other ear. Thus, single axons of different peripheral origins and different sensitivities to sound frequency form an orderly representation of ITD through the depth of nucleus laminaris.

The mechanisms underlying IID processing are not as well understood, either theoretically or empirically. Nevertheless, it has been recognized for many years[8] that there is neural sensitivity to IID. Most commonly, excitatory responses to acoustic stimulation of one ear are inhibited by stimulation of the other ear. The final level of the response is determined by the strength of excitatory and inhibitory input to each neuron. This, in turn, is determined partly by the intensity of the stimulus delivered to each ear and partly by factors intrinsic to the brain. For some neurons, inhibition dominates and may be complete when sound of equal intensity is presented to the two ears. For other neurons, sound presented to the inhibitory ear may need to be 20–30 decibels more intense than sound presented to the excitatory ear before any response suppression is observed.

In the ventral nucleus of the lateral lemniscus of the barn owl, Manley, Köppl and Konishi[2] show that neurons are organized topographically according to the IID for which their response is equally influenced by the excitatory and inhibitory ears (*c* in the figure). In the dorsal region this occurs for intensity differences where the input from the contralateral ear is more intense, and in the ventral region it occurs when the ipsilateral stimulus intensity is higher. Other systematic variations in response parameters across the dorso-ventral extent of the nucleus are correlated with this topography: excitatory and inhibitory response strengths and thresholds; spontaneous discharge levels; and sharpness of IID sensitivity. The results suggest that simple modulation of these parameters, particularly inhibitory response strength, can explain the observed topography.

Sound localization by humans and other



a, The physical cues for sound localization are established by a difference in the path length (*l'*) for sound reaching each ear, resulting in an ITD, and by the sound-shadowing effect of the head and outer ear producing an IID. Two cycles of a tone stimulus produced at the sound source (S) are shown as they would be recorded by microphones placed in the position of the ears. *b*, Schematic representation of axons from the left and right cochlear nucleus (CN) interdigitating within the barn owl nucleus laminaris (NL). Physical ITDs are offset by the time taken for spikes to travel through the depth of the NL from each side. According to their depth within the nucleus, the NL neurons shown receive coincident spikes from the input axons at different ITDs. Arrow, direction of electrode penetrations made by Carr and Konishi[1]. *c*, Electrode penetration (arrow) through the posterior part of the ventral nucleus of the lateral lemniscus (VLVp), showing (right) the responses to IID of neurons recorded at three depths. The IID at which balanced excitatory and inhibitory influences for each neuron was obtained is indicated. In dorsal VLVp, this IID is obtained when the contralateral ear intensity is higher (contra ↑). In ventral VLVp, the intensity at the ipsilateral ear needs to be higher (ipsi ↑) to give balanced excitation and inhibition (from ref. 2).

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terrestrial mammals is almost certainly more complex than in the barn owl. Instead of acting over the same, relatively restricted frequency range to code for azimuth and elevation, time and intensity differences between the ears of mammals provide cues over different portions of the audible frequency range and both seem to be predominantly used for coding stimulus azimuth. Elevation cues in mammals are thought to result from sound–spectral transformations by the head and by the pinna, the visible part of the outer ear[9]. In fact, information derived from just one ear can, under certain stimulus conditions, lead to sound localization performance in humans that is as accurate as that achieved using two ears[9].

Nevertheless, several key nuclei in the mammalian auditory pathway bear striking anatomical and physiological similarities to those in the barn owl known to be involved in localization. A topographic representation of IID has been demonstrated in the midbrain of the cat, for example, and two-dimensional neural 'maps' of auditory space have been found in homologous brain regions of several owl and mammalian species[10]. It therefore seems likely that at least some of the mechanisms for processing binaural sound localization cues are common in many vertebrate species. □

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Ocean Drilling Program

Breakup of Gondwanaland

*Leg 122 shipboard scientific party**

OLD, sediment-starved, passive continental margins are important for elucidating the thermal and structural processes that lead to rifting and continental breakup. These regions also contain clues about global palaeoenvironmental and climatic changes, as well as the record of sea-level fluctuations. There have been few deep-sea drilling studies of the older parts of continental margins even though their unique record reaches back to the Jurassic and Triassic, when there was only a single continental mass — Pangaea. Pangaea was split subsequently by the Tethys Ocean into Gondwana in the south and Laurasia in the north. We have studied the old Gondwanan passive margin of Exmouth Plateau, off the coast of northwestern Australia, to elucidate the evolution of rifting and drifting, in addition to the environmental changes that accompany and follow such momentous events.

The Exmouth Plateau is a rifted and deeply subsided fragment of continental crust, covered by more than 8 km of sediments, including nearly 1–2 km of post-breakup sediments. The present configuration of this region was initiated in the Late Triassic to Early Cretaceous (230–130 million years ago, Myr) by East-Gondwanan rifting between northwestern Australia, greater India and other Gondwanan fragments to the north. Leg 123 data suggest that whereas the northern margin of the Exmouth Plateau bordering the Argo Abyssal Plain had rifted before the Late Berriasian (140 Myr), the western margin bordering the Gascoyne Abyssal Plain developed during the late Valanginian (135 Myr) when sea-floor spreading was initiated there.

We drilled at six sites (Fig. 1). Four of these (759–761 and 764) on the Wombat Plateau yielded a composite record of sediments deposited during rifting in the Triassic (248–213 Myr) and during the Cretaceous (144–65 Myr) and Cenozoic (65 Myr to the present) following the breakup. The other two sites (762 and 763) reveal the Cretaceous to Cenozoic record of palaeoenvironmental and passive margin evolution of the western part of the central Exmouth Plateau. This record consists of a thick clastic shelf-margin wedge that prograded from a southern source area during the Early Cretaceous late-rift phase, and was there overlain by an Upper Cretaceous to Cenozoic pelagic (marine) sequence.

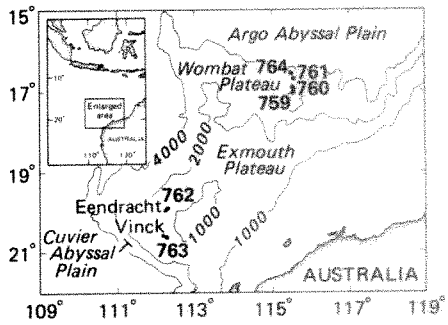


Fig. 1 Leg 122 sites (●) on the Exmouth Plateau. Commercial well sites are also indicated (•). Bathymetry given in corrected metres. (After von Stackelberg, U. *et al. BMR J. Aust. Geol. Geophys.* **5**, 113; 1980.)

Deep crustal extension and thinning of the northern Exmouth–Wombat Plateau area occurred during the Permian (286–248 Myr), and was followed by rifting during the Triassic. Carbonate rocks were deposited on the Wombat Plateau during the mid-Carnian (in the Triassic, around 228 Myr), in a southern embayment of a shallow Tethys Sea. The plateau periodically re-emerged until the Rhaetian (ending the Triassic, 213 Myr) when it developed a fully marine carbonate sequence. These carbonates resemble their coeval strata in the western Tethys region of the Alps. ▶

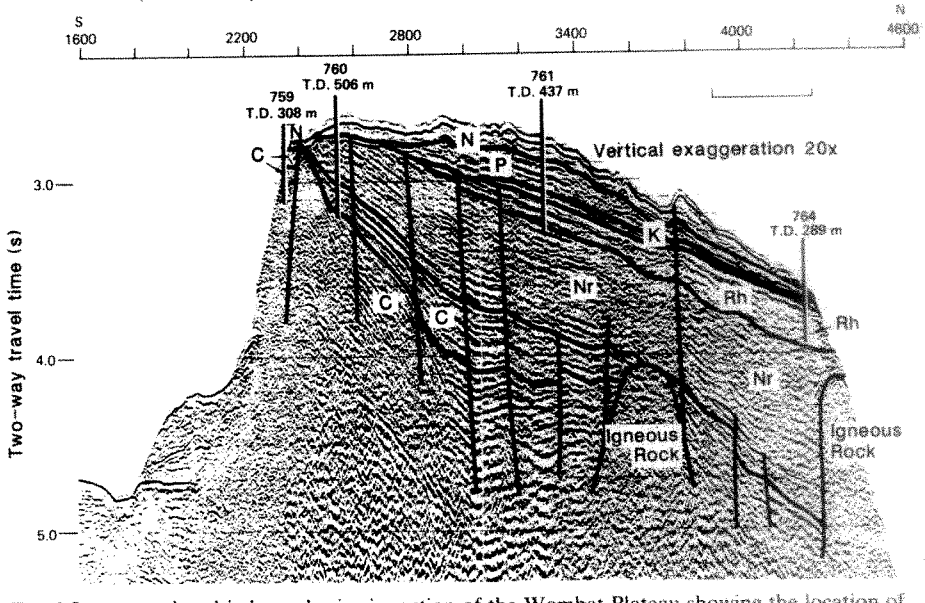


Fig. 2 Interpreted multi-channel seismic section of the Wombat Plateau showing the location of sites 759–761 and 764. Letters within the seismic profile refer to ages as follows: N, Neogene; P, Palaeogene; K, Cretaceous; Rh, Rhaetian; Nr, Norian; and C, Carnian. Scale bar, 10 km.

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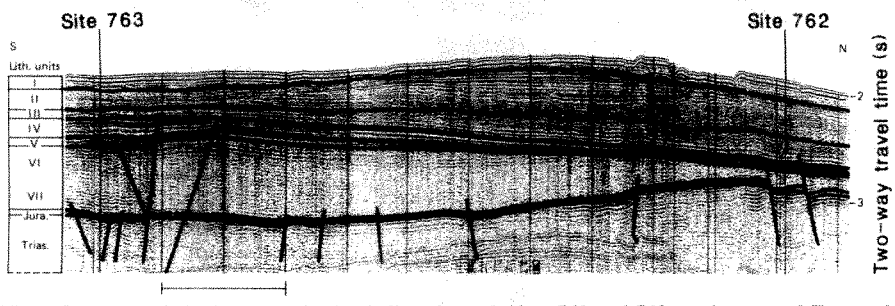


Fig. 3 Interpreted single-channel seismic line through sites 762 and 763 on the central Exmouth Plateau. Note thinning of the clastic wedge (units VI and VII) and thickening of the lower Tertiary sequence (unit II) from south to north. Scale bar, 7 nautical miles.

During the Jurassic (213–144 Myr), northward tilting raised Wombat Plateau above sea level again, and non-deposition or erosion prevented the preservation of Jurassic sequences before the onset of sea-floor spreading in the Argo Abyssal Plain. The plateau subsequently subsided rapidly during the early Cretaceous.

The time of breakup of the western and southern margins of the Exmouth Plateau is constrained by a major erosional unconformity, to around the end of the Neocomian (131–119 Myr), which agrees well with dating from sea-floor magnetic anomalies. Intervals of rising water depth are represented by condensed sections of thin glauconitic limestones and belemnite-rich mudstones. During the mid-Cretaceous (119–91 Myr), when the southern hinterland was drifting north-west with greater India, the southern supply of eroded rocks was cut off. Cyclic deposition of deep-water claystone during the middle to late Aptian (around 115 Myr) marks the onset of hemipelagic deposition (the 'juvenile ocean' stage).

A stagnant period of oxygen depletion produced a well developed black shale with up to 15 per cent organic carbon at the Cenomanian/Turonian boundary (91 Myr). Post-Cenomanian deposition became dominated by pelagic carbonate deposition, continuous in the northern part of the basin (site 762) and reaching site 763 to the south in the middle Eocene (around 50 Myr).

After isolating unconformities caused by tectonic events, we used a sequence stratigraphic approach to deciper sea-level fluctuations at each site. Our preliminary results indicate that there are important sequence boundaries on the Wombat Plateau between sediments representing the middle and late Carnian, at the Norian/Rhaetian boundary and in sediments of latest Rhaetian age, whose timing conforms well with global changes. Sequence boundaries recognized in the Lower Cretaceous of the central Exmouth Plateau also correlate well with global changes but indicate that the pattern of accumulation of deposits may need to be modified in regions of fast sediment supply. □

Evolutionary biology

Seed of destruction

H. C. J. Godfray and Paul H. Harvey

WHEN Jack Werren and his colleagues attempted[1] to select for the sex ratio in *Nasonia vitripennis*, a parasitoid wasp that attacks houseflies and related species, they found to their surprise that females producing only sons started to appear in the line being selected for male-biased sex ratios. They established by crosses using genetically marked flies that the trait (*psr*, for paternal sex ratio) is extra-chromosomally inherited. This was the first recorded occurrence of cytoplasmic inheritance through the paternal line of an animal. In their most recent work[2,3], these authors establish the nature and mode of action of *psr*, and provide an explanation for its evolutionary origin. The serendipitous discovery of what has been termed the most selfish genetic element that has yet been described offers the prospect of new insights into the structure and function of eukaryotic chromosomes.

Initially, it was uncertain whether *psr* was an infectious venereal disease which causes the death of female progeny, or a cytoplasmic factor transmitted to the egg during fertilization which causes it to become male. *N. vitripennis*, like other hymenopterans, has a haplodiploid genetic system with haploid males developing from unfertilized eggs and diploid females from fertilized eggs. As with many other hymenopterans, the female wasp produces a male-biased sex ratio when her sperm reserves are depleted, and a female-biased sex ratio in circumstances where her progeny tend to mate among themselves[4,5] or when an appropriate maternally inherited cytoplasmic sex-ratio factor is present[6]. The proportion of progeny carrying the *psr* trait was highest when a female-biased sex ratio was expected[5]. It therefore appeared that *psr* enters the putative female eggs at fertilization, converting the zygotes to males.

Cytological examination revealed that sperm carrying the *psr* trait enter and fertilize the egg as normal. During the first mitotic division, however, half the chromosomes appear clumped together in a dense mass of chromatin and do not participate in further cell divisions[2]. The embryo, with its now haploid chromosome complement, develops as a normal male wasp. Genetic marker studies reveal that it is the paternal genome which is eliminated.

Extra chromosome

In their new work, Nur, Werren and co-workers reason that *psr* might be discovered by identifying the DNA present in infected but not in uninfected males. They created a λ-phage genomic library from *psr* male DNA, and searched for clones that hybridized with radioactively labelled whole DNA from *psr* males but not with DNA from normal males. They found 36 such clones, and subjected several of them to restriction-enzyme analysis. It turns out that much of the *psr*-specific DNA is in the form of satellite-like, short tandem repeats, the most abundant sequence being 171 base pairs long. This finding is not compatible with the idea that *psr* is a virus or a transposon, but suggests that it could, after all, be a supernumerary chromosome because satellite-like DNA is characteristic of heterochromatized regions of chromosomes. Indeed, detailed cytological study reveals a small chromosome found only in *psr* males.

Supernumerary, or B chromosomes are fairly common in animals and some even show varying degrees of meiotic drive[7]. But the devastating selfishness of *psr* is unique: in a mating between a male bearing *psr* and a female, *psr* first destroys the chromosomes of its original host and then, in the next generation, those of its newly adopted host. Though much of the DNA in the B chromosome is in the form of tandem repeats, specific regions are known to code for a *trans*-acting factor involved in the super-condensation of the parental chromosomes. Analysing the mode of action of *psr* could reveal the mechanism of chromosome condensation, which is poorly understood.

Where does the B chromosome come from and what is its fate? There are now preliminary answers to both questions[8]. There are several cytoplasmically incompatible strains of *N. vitripennis*, and in matings between wasps from two such strains, the paternal chromosomes are broken down. But is is possible to recover fragments of partially destroyed chromosomes that display high transmissability through the male line but much lower

transmissability through the female[9]. Such a fragment is under strong selective pressure to change the sex of the female zygotes it normally finds itself in to more congenial male zygotes.

Method of spread

Once *psr* has evolved, it may spread. Werren points out[10] that if *psr* is transmitted by all the sperm of an infected male in a spatially unstructured population, the equilibrium frequency of *psr* is $(2x-1)/x$, where x is the proportion of eggs that female wasps fertilize. This means that *psr* will not persist unless $x > 0.5$, that is, unless female wasps fertilize more than half their eggs, which would mean producing a female-biased sex ratio in an uninfected population. *N. vitripennis* does indeed do this because its populations are spatially structured with mating occurring among the offspring of small groups of females, with consequent very high levels of local mate competition[11,12]. Although this female-biased sex ratio favours the spread of *psr*, another feature of spatially structured populations tends to hinder its spread: if all the females laying their eggs in a single patch (perhaps a localized population of housefly larvae) have mated with *psr* males, all their progeny will be sons who will die without mating. Theoretical models of the spread of *psr* in spatially structured populations indicate that persistence is possible only for intermediate levels of population subdivision[13].

Ironically, the persistence of *psr* may be markedly enhanced by the presence of maternally inherited cytoplasmic factors that cause female-biased sex ratios[13], two of which are known from *N. vitripennis*[6,14]. No systematic search for extrachromosomal sex-ratio factors has been undertaken in Hymenoptera other than in *N. vitripennis*. Of all groups with variable sex ratios, parasitic wasps were thought to be the best understood: perhaps there is a whole new level of complexity yet to be explored. □

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Raymond Arthur Dart (1893–1988)

RAYMOND Dart died in Sandton, Johannesburg, on 22 November 1988, almost 64 years to the day since he had received the chunk of sandy limestone containing the Taung skull that was to create his reputation. Dart recognized certain extraordinary features of the skull — its relatively erect poise, small canine teeth and endocranial capacity, scarcely differing from those of extant apes. But he noted also that the form of the brain was human-like in its parietal lobe. In particular, he recognized the impression of what he took to be the lunate sulcus. It was well back, as in modern man, and not well forward as in modern apes. He then made a great intellectual leap: he interpreted this curious blend of traits as evidence for the former presence in Africa of an anthropoid that had moved substantially in a human direction. This he claimed in his historic paper in *Nature* on 7 February 1925. He named the species *Australopithecus africanus*.



Associated Press/Univ. Witwatersrand

Dart — effected a revolution in knowledge.

Many deterrents to the acceptance of Dart's claim were predicated on the accepted wisdom of the day: the idea that Asia, not Africa, had cradled mankind; and the notion that the brain had expanded early in hominization, a view supported by the features of the ill-famed Piltdown bones. Moreover, the Taung specimen was of a child and, it was averred, the nature of the adult could not have been predicted. Arthur Keith, with most later investigators, rejected the evidence of the lunate sulcus, holding that its position could not be identified with certainty on the Taung endocast.

The main controversy Dart stirred up did not abate for 25 years, when many new specimens from the Transvaal were compared with the skeletons of modern apes. It was realized that, even if one discounts the lunate sulcus, enough other features mark the specimen not only as unique, but as morphologically intermediate in several respects between apes and men. Only then did it emerge that Dart's claim had been basically correct and, indeed, that there was every justification for classifying the fossils as members of the Hominidae.

Dart's recognition and interpretation of the significance of *Australopithecus* remains one of the great steps forward in the history of palaeoanthropology. With hindsight, the essence of his 1925 contribution lay in his willingness to overlook the small brain size of *Australopithecus*, in the face of other strong pointers to its evolutionary status. Brain-size alone, he declared in a 1956 paper, was not the acid test; the form of the brain was more important. Late in life, Keith, the first of Dart's main opponents, generously acknowledged that he had been wrong and Dart right: he proposed to call the australopithecines 'Dartians'.

After World War II, following discoveries of fossil baboons at Makapansgat, northern Transvaal, Dart was lured back into the field — and a new chapter opened. Intrigued by the thousands of fossilized, broken antelope bones in that cave deposit, he proposed in 1955 that the bones had been broken deliberately, fashioned and used by the australopithecines. His proposed Osteodontokeratic culture was based on the use as implements of bones, teeth and horncores, especially of ungulates. This notion has now been largely rejected, as evidence has built up that big cats, hyenas and porcupines were probably the bone breakers and accumulators.

But Dart's hypothesis had a remarkable spin-off. When he first proposed his bone-tool theory in 1955, scarcely anything was known of how animals, or agents like sunlight and water, affected bones after death. To disprove Dart's proposal, a host of new studies were made of the effects of such biotic and physical agencies on bones. A new scientific discipline came into being, called taphonomy. Dart's ideas were a major catalyst in the birth of this new field. Thus, it can be claimed of Dart that he effected a revolution in knowledge about man's place in nature, and that indirectly he triggered the foundation of a new scientific discipline.

Raymond Dart was born during the flood that swept Queensland, Australia, in 1893. He read science at the new University of Queensland, Brisbane, and medicine at Sydney. After serving in the Australian Army Medical Corps, he worked under Elliot Smith in the Anatomy Department at University College, London. There followed six months as a Rockefeller fellow in the United States. In 1923, be became head of the Department of Anatomy at the newly formed University of the Witwatersrand Medical School, a position he held for 36 years. Dart was a prime architect of the Medical School and a maker of men. The Raymond Dart Collection of Human Skeletons is one of the tangible monuments to his initiative and foresight. Phillip V. Tobias

Phillip V. Tobias is Professor of Anatomy and Human Biology at the University of Witwatersrand Medical School, Parktown 2193, South Africa.

Active galactic nuclei

Pointing towards the source?

Virginia Trimble

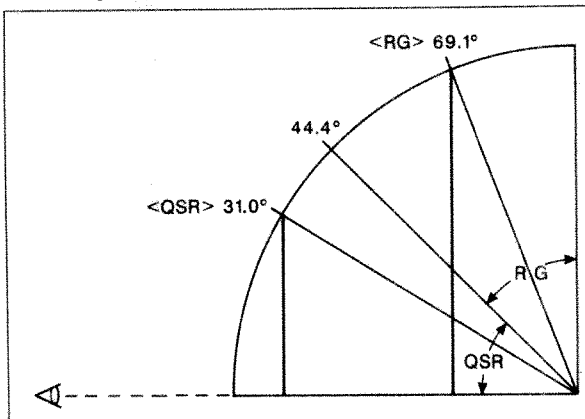
QUASARS, like beauty, may be in the eye of the beholder. So suggests Peter D. Barthel in a paper in the latest *Astrophysical Journal*[1] that collates observations collected by his[2] and many other groups of astronomers. According to his version of the unified scheme for active galactic nuclei, radio-emitting quasistellar objects (QSOs) and powerful radio galaxies are the same sorts of objects, seen from different directions, and radio-quiet QSOs may be the similarly oriented subset of powerful galaxies with bright infrared sources (called IRAS galaxies after the satellite that measured their fluxes).

The generic name, active galactic nuclei (AGNs), indicates a long-standing recognition of some kind of unity within the zoo. The beasts on display include (a) quasars (an attempt at pronouncing the acronym QSRS for quasi-stellar radio source), which are optically compact, show broad emission lines at large redshifts, and emit powerfully at radio wavelengths, (b) QSOs (much more numerous and not strong radio sources, but otherwise similar), (c) BL Lac objects (with rapid variability, strong polarization, and weak or no emission lines; named for their prototype), (d) radio galaxies, (e) optically violent variables (OVVs) and (f) Seyfert galaxies (bright nuclei and strong emission lines with moderate redshifts, named for their discoverer). The host galaxies are generally thought to resemble ellipticals for types (a) and (d), spirals for (b) and (f). Many are interacting galaxies.

All AGNs exhibit the astrophysical difficulty of producing lots of energy from small volumes with the capacity for very rapid change in both brightness and location of the emitting regions. And all are currently explained by a standard model[3,4]. Its components are a massive black hole (10^{5-10} times the mass of our Sun) at the centre of a galaxy; energy generation and transport by some combination of gas flowing inward and magnetic fields linking the hole to more distant gas; and one or two outgoing, well-collimated jets of particles, field and photons moving at speeds close enough to the speed of light that special relativity must be applied rather carefully to avoid inconsistencies in interpreting the observations[5].

The model clearly provides a wealth of parameters that can be varied among assorted kinds of AGNs — the mass of the black hole, the rate of gas inflow (relative to an upper limit set by radiation pressure), the field strength and configuration, the geometry of surrounding gas, the mechanism and precision of jet collimation, the dominant components of the jet (plasma of electrons and positrons or electrons and protons; magnetic field; photons), and the angle the jets make with our line of sight.

Earlier versions of a unified scheme[6-9] attached considerable importance to the last, orientation, parameter. A jet seen more or less end-on was invoked to explain several phenomena that are common, but by no means universal, in quasars, including rapid flux variability and rapid outward motion of radio-emitting blobs along the jet direction (called superluminal motion because a naive analysis of it that neglects the effects of orientation and special relativity leads to apparent motion at velocity = 2–10 c). Such a picture successfully organizes a variety of observed features of quasars and their ilk.



Orientation of the approaching jet of active galactic nuclei relative to the observer's line of sight. The dividing line between quasi-stellars and radio and infrared galaxies occurs at about 44°. Thus the average radio galaxy ⟨RG⟩ at 69° should have an apparent, projected radio diameter about twice that of the average quasar ⟨QSR⟩ at 31° as seems to be the case. (Courtesy of P. D. Barthel.)

The classification of some quasars as roughly end-on jets and some as roughly side-on, runs into a couple of difficulties, however. First, presence of superluminal motion should be correlated with small angular size of extended radio emission as projected on the sky. It is not. The paper by Barthel *et al.*[2] in fact reports apparent expansion at 2.5 c for the core of the quasar 4C 34.47, which has the largest projected size of any known (560 kiloparsecs assuming a Hubble parameter H = 100 km s^{-1} Mpc^{-1}). Second, the radio flux we see should be enhanced by beaming and the X-ray flux should not; yet the two are rather well correlated. It is also sometimes hard to understand the non-detection of the expected, diametrically opposed, jet aimed away from us.

In Barthel's restructured scheme (see figure), all quasars are beamed within 44° at us (average, 31°), and the otherwise-directed objects are seen as powerful radio galaxies, whose projected sizes are indeed larger by about a factor of two. Radio-quiet quasistellar objects may be the corresponding end-on views of galaxies that we otherwise see as strong infrared sources (because dust absorbs and re-radiates much of the emission in the equatorial direction). The presence or absence of radio emission is then an intrinsic property of a particular hole–accretion–collimation combination, and X-ray emission must be somewhat enhanced by beaming effects. The Seyfert galaxies provide some supporting evidence. The two subtypes, Seyfert 1 and Seyfert 2 galaxies, have been attributed to pole-on and equator-on directions of view on the basis of other evidence[10]. And, indeed, Seyfert 1 galaxies are more often the strong X-ray emitters; and the most pronounced superluminal galactic source, 3C 120, resides in a Seyfert 1. Barthel suggests that all host galaxies will be found to be experiencing some degree of interaction with neighbours. The relationship with yet another kind of activity, also widely blamed on interactions and called star bursts (meaning bursts of rapid star formation), remains to be worked out.

Barthel provides eight additional predictions of his scenario, pertaining to polarization, optical emission line, counter-jet and infrared properties of the several classes of objects. He also proposes two kinds of observations that could rule it out — evidence for systematic differences in the morphology or in the environments between the host galaxies of quasars and radio galaxies, or between QSOs and infrared galaxies. Curiously, the definitive test of going around to the other side of a few and looking is not mentioned. □

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Dating techniques

History revealed from bones

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As Mark Antony pointed out[1], most of what we should like to know about our forebears lies interred with their bones. But it is no easy task to reconstruct the cultural life of our ancestors from the fragmented evidence that their bones (and other remains) provide. Bones are very complex structures and contain potentially a wealth of information about, for example, an individual's genetic constitution, medical history, environmental influences and cultural practices. They are also far from chemical equilibrium, and information is inevitably lost with the passage of time. The effect of the burial environment, in terms of diagenetic changes both to the organic and inorganic components of bone, is even more marked, the remaining information being overwritten and further scrambled. Nevertheless, progress continues in sifting out the effects of environmental contamination and in deciphering what remains. Some new papers in *Geochimica Cosmochimica Acta*[2-4] exemplify this, and reinforce research reported at a recent workshop[5].

Analytical work on ancient bone ranges from isotope studies to immunochemical detection of antigens, taking in trace-element measurements, amino-acid racemization, protein identification and the search for DNA. So far, isotope methods promise the most straightforward results. These include dating (^{14}C and, much more arguably, $^{230}Th/^{234}U$), and dietary information (^{13}C and ^{15}N). But even here the state of preservation of the bone is paramount.

Radiocarbon dating of bone, especially in the critical period of the transition of man from Neanderthal to modern, thought to have ended about 30,000 years ago, can produce dates far too young because of a small admixture from younger environmental contamination. How this admixture might be separated depends on our understanding of the way in which unknown contaminant molecules can crosslink to degraded bone collagen. The same problems apply to stable isotope results; as carbon and nitrogen atoms pass along the varied food chains of man's diet, the different small degrees of isotope fractionation can be recognized. Distinctions between marine and terrestrial animals, leguminous and non-leguminous plants, as well as plants growing by different photosynthetic pathways, are easily made, and more subtle influences of environment (such as climatic stress or the effect of famine) are now being explored. But the measurements are subject to the relatively inscrutable effects of bone diagenesis.

In something of a technical *tour de force*, Stafford *et al.*[4] have now isolated individual amino acids from bones in varying states of decay and have measured their radiocarbon age by accelerator mass spectrometry. As expected, the results are complicated, but the admixture of environmental amino acids can now be clearly followed. In a mammoth bone known to be about 11,000 years old, the dates obtained for some of the amino acids were only 3,000–4,000 years old.

The new work of De Niro and Weiner[2,3] aims to develop strategies to recover collagen fragments free from contamination. One approach is to exploit subtleties in the biomineralization of the hydroxyapatite/collagen matrix of the bone, in which a small fraction is in the form of multicrystalline arrays. This fraction is preserved after the treatment of the matrix with sodium hypochlorite, and in fossil bone still retains isotope signatures much closer to those expected for the original bone. Non-collagenous proteins and lipids are also apparently better preserved in these 'aggregates'.

A second approach that is being explored is to take advantage of the characteristic sequential structure in collagen: glycine–Y–X, where Y is often proline or hydroxyproline and X is any residue. This structure is specifically cleaved by the enzyme collagenase into fragments of relative molecular mass typically 500–700, which can then be extracted and purified. Although yet to be proved, the benefits for the radiocarbon dating of bone, particularly from hotter regions than northern Europe, are potentially very significant. Much Middle Eastern chronology, for example, rests on dating of unsatisfactory charcoal samples; the collagen levels surviving in bone are usually too low to provide reliable dates by existing techniques. Reliable dates obtained by these new techniques could transform our understanding of some of the archaeology of the region.

Other dating methods are less likely to evade the effects of environmental influence[5]. Uranium-series dating, for example, depends on modelling the uptake of uranium ions by buried bone from the ground water. Even the basic question of why some bones seem better preserved than others found in the same stratum on a site is not yet easy to answer. Perhaps the first steps are being taken in the continuing study of the disintegration of macromolecules from naturally weathering bones in the Amboseli National Park of East Africa. The first few years of a bone's death may be crucial for preserving its information for posterity. □

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Circadian rhythms

Mutant hamster in a hurry

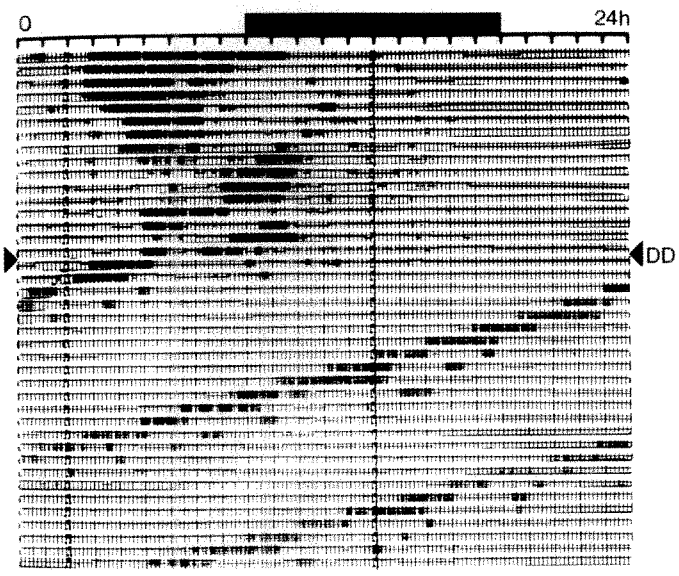
N. Mrosovsky

THE golden hamster (*Mesocricetus auratus*) is to chronobiologists what the fruitfly is to geneticists. Hamsters occupy this niche because their circadian rhythms of wheel-running activity are easy to measure, easy to quantify and highly predictable. At a recent meeting*, Martin Ralph (previously at the University of Oregon, now at the University of Virginia) reported his new work[1] on how the circadian-activity rhythms of hamsters can be experimentally manipulated by transplanting a part of the brain.

When the light–dark (LD) cycle is 14–10 hours, wheel-running in this nocturnal species is neatly confined to the hours of darkness. An occasional individual displays a sloppy or unusual rhythm, and such animals are usually discarded from experiments. But when Ralph noticed that one hamster started to run in its wheel several hours before the onset of darkness in a 14–10-hour LD cycle (see figure), he placed the animal in constant darkness, reasoning that the early activity onset in the presence of a LD cycle might reflect a circadian pacemaker with an unusually fast frequency. In this condition the hamster's activity free-ran with a periodicity of only 22 hours. The shortest free-running rhythm previously recorded[2] by Ralph and Menaker, out of more than 1,000 hamsters tested, was 23.5 hours.

Tests on these offspring revealed that there is a trait, an abnormally short free-running circadian period (*tau*), at a single autosomal location[2]. The *tau* mutant can thus be used to tackle unresolved questions about biological rhythms. For example, it is thought that the supra-

* Society of Neurosciences, Toronto, 13–18 November 1988.



Raw data showing the discovery of the mutant *tau* hamster. Each line represents 24 hours. Successive days are mounted beneath each other. Dark pen marks indicate periods of wheel-running activity; dark bar at top, time of darkness on a light–dark 14–10-hour schedule; arrows, change of lighting schedule from light–dark 14–10-hour to continuous darkness (DD). (Courtesy of M. Ralph.)

chiasmatic nuclei (SCN) in the brain are the site of circadian pacemakers in mammals, but this assertion has not been proved. True, lesions in this area abolish circadian rhythms of activity and other variables[3], but this does not prove that a pacemaker resides there. It could simply mean that the integrity of this area is necessary for the expression of rhythmicity. Transplanting fetal SCN into animals whose own SCN have been ablated can result in restoration of rhythms. This again does not prove that the SCN contain a pacemaker. The circadian machinery might be elsewhere, with the SCN simply providing some input necessary for its functioning. What is required is a demonstration that transplants produce rhythms that have characteristics which are specific to the clock of the donor.

In sparrows, removal of the pineal results in a loss of circadian rhythmicity. Menaker and his co-workers have previously shown that transplants of the pineal gland restore rhythmicity to pinealectomized sparrows, but also that the phase of the donor's rhythm determines the phase of the recipient's rhythm[4]. Thus, the time of day at which the recipient sparrows become active depends on whether the donor birds have been kept on a normal or a reversed LD cycle. This still is not quite definitive because the phase of a rhythm is a temporary characteristic that can be quickly shifted in various ways. It would be more convincing to transplant a persisting stable characteristic of a clock such as the speed with which it runs. In sparrows, however, there is too little inter-individual variation and too much intra-individual variation to make transplanting periodicity convincing.

There have been some attempts to transplant periodicity between strains of rats with different clock speeds, but activity rhythms in rats are generally not as clear as those in hamsters. A hamster mutant with a period outside the range of those for the wild-type is the ideal subject for this kind of work.

Ralph's new data show that the period of the circadian-activity rhythms of wild-type hamsters can be shortened first by ablating their own SCN and then by inserting fetal mutant SCN. Conversely, the period of mutant hamsters can be lengthened by replacing their own SCN with fetal wild-type SCN. This result is the confirmation of the pacemaker role played by the SCN. It is also a striking example of the transplantation of a behaviour.

The mutant (*tau*) hamster is likely also to provide insight into other questions about biological rhythms. Attempts to discover the gene product of this single locus and to unravel its molecular biology have already begun. It will be interesting to make comparisons with the fruitfly period (*per*) gene, which has already been cloned and sequenced (see the recent discussion in News and Views[5]). Understanding fundamentals about rhythmicity will eventually help in ameliorating rhythm disturbances such as those occurring in jet lag, shift work and some types of affective disorder. A nice touch is that Ralph and Menaker have rejected the option of trying to patent the mutant hamster. Altogether, this is textbook science at its best. □

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Daedalus

A reducing diet

COOKING is not a consciously chemical discipline. Only by trial and error have chefs learnt to exploit the hydrolysis and denaturing of proteins, the partial oxidation of fats, and the Maillard reaction between proteins and sugars, to produce their wide palette of sumptuous and tempting flavours. It is largely oxygen that develops the subtleties of taste, by forming the delicate traces of higher aldehydes, ketones and esters that impart flavour; but over-oxidation gives sour, acrid fatty acids. So cooking limits oxidation: typically by careful temperature control, or (as in frying and boiling) by submersion.

Modern industrial food processing uses anti-oxidant additives; but Daedalus has a more wholesome approach. The traditional chef has to join his battle with oxygen over an open stove, and faces rancid, burnt or over-cooked disaster if he loses it. But modern electric and microwave ovens are quite independent of air. So DREADCO's chefs are cooking all sorts of dishes not in air, but in a non-oxidizing atmosphere of nitrogen or argon.

This new sort of cooking has many benefits. Very high temperatures can be explored under full control: the food cannot burst into flame. Controlled amounts of oxygen can be introduced at crucial stages, to promote the development of new and subtle flavours without going too far: just as wine develops its subtle bouquet from the slight leakage of oxygen into the bottle through the cork, but is spoiled if too much gets in. Gourmets should revel in the new and unexpected flavours that will thus be teased out of quite ordinary foodstuffs. Dull and obvious products like lentils and rhubarb and corned beef may come alive with subtle delights; hidden virtues may be revealed in marrow, tapioca, and the British sausage; the range of delightful complementary pairs, like eggs and bacon, and fish and chips, may be wonderfully extended. Culinary anaesthetics like salty gravy and sugary custard may no longer be needed. Even hopeless raw materials like lettuce and battery chicken may at last be made to taste of something.

More boldly still, the DREADCO chefs are filling their new ovens with frankly reducing atmospheres: hydrogen, carbon monoxide, even ammonia. The entirely novel flavours thus developed may be so strange and amazing as to be positively overwhelming. The hydrogen-reduction oven may be best employed as a 'decooker' to rescue overdone cooking. By converting the sour fatty acids of over-oxidation back to flavoursome aldehydes again, an over-cooked joint, deadburnt cake or pyrolysed pie might be reclaimed for the gourmet's table.

David Jones

Safety of Cameroonian lakes

SIR—In dealing with lethal releases of CO_2 from lakes, the people of Cameroon require firm scientific evidence on which to base decisions for evacuation and disaster control. We are concerned, therefore, by the misleading and speculative letter by Pourchet *et al.*[1] stating "it is likely that many of the crater lakes (in Cameroon) have emitted gas bursts, or will do so". Our recently completed studies suggest that gas bursts from other lakes in Cameroon are, in fact, unlikely.

The accumulation and massive release of CO_2 gas from crater lakes is unique, and has been documented only for lakes Nyos and Monoun in Cameroon[2,3]. The circumstances required for this phenomenon to occur are very rare. There must be an adequate gas supply, such as the CO_2-charged soda springs occurring in Cameroon, and this supply must be trapped by surface deposits or by a deep, strongly stratified lake. There is also good evidence that a build-up of CO_2 and solutes in bottom waters occurs long before any gas burst[2,4]. Our recent limnological survey indicates no such premonitory build-up in any Cameroonian crater lake apart from Nyos and Monoun[4,5].

The argument of Pourchet *et al.* seems to rest on two points. The first is that porewaters and bottom waters of Lake Bambuluwe are "close to gas saturation at the sampling depth (58 m)". But observing gas bubbles in a core brought to the surface, as they did, indicates little more than oversaturation at pressures (below 5.8 atmospheres) greatly reduced from those found in the bottom sediments. Such gas pressures are common in freshwater sediments; indeed nearly all of the sediment cores we have raised from 11 different crater lakes in Cameroon degassed at lake surface pressures.

The second part of their argument links the homogeneous distributions and higher than expected concentrations of ^{210}Pb in sediment cores from lakes Nyos and Bambuluwe. Our sediment cores from lakes Nyos and Monoun were mixed rapidly because of violent degassing at the surface. Pourchet *et al.* do not detail their methods, but only *in situ* sampling or freeze-coring would maintain stratigraphic integrity of the sediment in a gas-charged lake. In Lake Bambuluwe, the uniformly high ^{210}Pb concentrations they report for the upper 15 cm have other explanations. For example, a 1m core taken by us in 1985 revealed an upper 15–20-cm layer of clastic material overlying darker micaceous mud. This surface layer may correspond to the recently built access road around the lake. Such watershed disturbances are known to increase the flux of radiogenic lead to lakes[6]. We believe that the reported similarities between sediment ^{210}Pb in Lake Nyos and Lake Bambuluwe may be spurious, and thus inappropriate for use as predictors of future gas bursts.

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Dominance of South American marsupials

SIR—The discovery of mammal remains from the early Cretaceous of Cameroon by Jacobs *et al.*[1] is of unquestioned importance in interpreting mammalian evolution and biogeography, particularly regarding the key transitions to metatherian and therian 'grades'. The statement by Jacobs *et al.* that metatherian marsupials represent "the dominant living mammals of South America and Australia", is surely coloured by a geological perspective, however.

Although the ecological and evolutionary dominance of Australian marsupials during the Tertiary and Quaternary seems well founded, this is not true of today's American marsupials, despite their remarkable diversity and radiations in the Tertiary[2,3]. Modern New World marsupials comprise only three families, 15 to 19 genera, and 85 species, most endemic to tropical South America[4]. At one comparatively well-studied site in south-eastern Peru (Cocha Cashu in Manu National Park), nine species of marsupials comprise 9 per cent of the known mammal fauna — there, four mammalian orders (Rodentia, Chiroptera, Carnivora and Primates) exceed the familial, generic and specific diversities of marsupials, and Manu records of bats and rodents are incomplete[5]. Elsewhere, 34 genera and 49 species of bats have been recorded at a single locality in Rondônia, western Brazil (specimens in the Museu de Zoologia, Univ. São Pauolo), and that country supports at least 7 families, 53 genera and 183 nominal species of rodents[6].

Although 12 mammalian orders occur in South America, 43 per cent of its mammal species are rodents, including at least 250 species of Sigmodontinae (Muridae)[7]. In terms of diversity and abundance, rodents and bats greatly outstrip marsupials as the most dominant orders of modern South American mammals. Indeed, the contrast in diversity and dominance of marsupial lineages before and after the "Great American Interchange" has been an important focus for studies of faunal dynamics and turnover.

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Black holes dispute

SIR—Gunzig, Géhéniau and Prigogine[1] attempted a numerical evaluation of the inflationary model of black hole production postulated[2] some time ago. Unfortunately, their paper contains several errors. We emphasize that the principal hypothesis[2] is not destroyed as several modifications to the model of Gunzig *et al.* are not only possible, but likely.

First, the matching conditions of equations (14) and (15) of Gunzig *et al.* are stated to imply $\alpha \simeq \sqrt{\beta}$. In fact these conditions are not sufficient and the value of α is very sensitive to the input. Thus, for example, as β / C^2 varies from $(1 - \varepsilon)$ to $(1 + \varepsilon)$, where $\varepsilon \equiv 1 - \Delta_i \simeq 10^{-27}$, α varies from 0 to $\sqrt{\beta}$. Input must be fine-tuned to 27 decimal places to obtain the result of Gunzig *et al.*. It is equivalent to tuning a discontinuity in temperature between de Sitter and Robertson–Walker stages to the same precision.

Second, Gunzig *et al.* compute t_e using three helicity states. The present count, a lower bound, is 119. Using the model of Casher and Englert[2] to determine the de Sitter temperature (as we consider the determination of Gunzig *et al.* unreliable) the matching radius is reduced by a factor of 10^{-23}. But the hypothesis is not destroyed because the true turnover time is expected to be much greater than t_e.

Third, Gunzig *et al.* determine the mass density of the massy component of matter from the matching condition. To obtain the number density and thence the entropy per proton they divide this by the proton mass. But the matching temperature is $\sim 10^{17}\, m_{proton}$. So the protons (which are certainly in equilibrium) will be in the β/R^4 term of the energy source and not

in the α/R^3 term as Gunzig *et al.* suppose. Their calculation could be used to estimate the lepto-quark density of grand unified theories, but certainly not the density of any of the present constituents of matter.

We stress that the field-theoretical foundations of the production mechanism as well as the instability of Minkowski space are flimsy, at best — though not necessarily wrong — owing to the use of an unconventional and arbitrary subtraction procedure[2]. The model of inflation through black hole production[2] must be regarded as a hypothesis without solid theoretical support. If such exists, it must await the yet-to-come quantum theory of gravity. To our mind, any numerical evaluation, unless it definitely detroys the model, is premature. (A more detailed account of our calculations is available in ref. 3 or from us).

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GUNZIG REPLIES—Brout and Spindel claim that our paper[1] contains several "errors". We firmly disagree with this conclusion. I will consider their criticisms.

The first point concerns the matching parameters α and β. We agree entirely with their remark concerning the sensitivity of α to the input. But they miss the key point, which is that there is a subtle compensation of infinitesimals which leads to our result $\alpha/\sqrt{\beta} = 0(1)$: it appears indeed that the solution of the matching equations (14) and (15) is given by $\alpha/\sqrt{\beta} = 1-\varepsilon/\varepsilon_1$, where $\varepsilon = (\beta/C^2)-1$ and $\varepsilon_1 = 1-\Delta_1 \approx 10^{-27}$. It results straightforwardly from the definitions of the parameters β and C, that $\beta/C^2 = 1/\Delta_1\ (T/T_{BH})^4$. The value of T/T_{BH} is easily evaluated in the euclidean parameterization of the joining conditions; the latter is appropriate as the joining point belongs to the de Sitter asymptotic region. It appears that $(T/T_{BH})^4 = 1-\varepsilon_2$ with $\varepsilon_2/\varepsilon_1 = 0(1)$. Hence,

$$\alpha/\sqrt{\beta} = [1-\frac{(\varepsilon_1-\varepsilon_2)}{\varepsilon_1}] = \frac{\varepsilon_2}{\varepsilon_1} = 0(1)$$

as we announced[1]. Moreover, when the cosmogenesis model is wholly reformulated in the framework of euclidean Robertson–Walker parameterization, as we did recently[4], the matching conditions give rise to much simpler algebraic relations than those associated with the open Robertson–Walker parameterization. These relations lead straightforwardly to conclusions identical to those resulting from the $\approx \sqrt{\beta}$ open condition.

The second point concerns the number of helicity states involved in our considerations. We mentioned this problem at the time[1]. Since then, we have shown[4] that the inclusion of any higher number of helicity states leaves all the results unaltered, provided that the black hole mass M is increased (by a factor of about 5 for $N = 100$, for example).

The third criticism concerns the use of the proton mass in the evaluation of the specific entropy. First of all, as mentioned in our paper[1], the fundamental quantity to be considered is the adiabatic invariant $(1/T)\ (\varrho_\gamma/\varrho_m)$ which does not involve any elementary particle mass at all. Our calculations produce the correct experimental value for this quantity as well. Nevertheless, the quantity which is traditionally discussed in the literature is the specific entropy per proton. To obtain the latter, it is fully legitimate in our context to use the proton mass. Indeed our joining procedure connects directly the de Sitter stage to the present cosmological stage. We mentioned in our conclusions that in a more realistic model, our parameters α and β would be time-dependent.

In addition to these three specific criticisms, Brout and Spindel are pessimistic about the field-theoretical foundations of the production mechanism as well as the instability of Minkowski space. These remarks are directed towards the series of papers on the subject, initiated by Brout, Englert and myself[5]. I share this concern. It is for this very reason that we have now developed[4] a phenomenological approach which eliminates the field theoretical subtleties. In this context, simple thermodynamical arguments render the explicit use of field-theoretical considerations no longer necessary.

In conclusion, Brout and Spindel have missed the main points of our paper. These involve the reinterpretation of Einstein's equations leading to a burst of entropy associated with matter creation in the early stages of the Universe.

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Selective action of artificial membranes

SIR—Rubinstein *et al.*[1] claim to have prepared the first example of a stable, ion-selective artificial membrane of monolayer thickness, which successfully mimics basic structural and functional properties of natural bilayer membranes. Their membrane was prepared on a gold electrode by coadsorption of two components from bicyclohexyl/chloroform solution. After transfer to an aqueous solution, the first of these (OM) apparently acted as a blocking agent, and the second (TBEA) as an 'active' ligand forming 1:1 complexes with divalent metal ions, such as Cu^{2+}, but not with travalent ions, such as Fe^{3+}.

To establish that their 'artificial membrane' was working as they claimed, the authors needed to prove that the only observed electrochemical reaction was caused by the active ligand chemically distinguishing between Cu^{2+} and Fe^{3+} ions in solution. Although certain evidence was adduced in favour of this hypothesis, we believe that there is an alternative and much simpler interpretation of the experimental results in which the active ligand has no role.

When the 'artificial membrane' was placed in a solution of Cu^{2+} and Fe^{3+} ions, Rubinstein *et al.* observed no Cu^{2+} 'underpotential' (non-bulk) and Fe^{2+}/Fe^{3+} peaks; repression of the peak for bulk deposition of copper onto the electrode by ~ 250 mV; and a loop in the cyclic voltammetry. Rubinstein *et al.* interpret these observations as follows: Cu^{2+} peaks were absent because copper atoms were deposited inside the 'artificial membrane' at some distance from, rather than directly on, the electrode surface; the lack of Fe^{2+}/Fe^{3+} peaks was due to a lack of sites where Fe^{3+} ions could bind; and the shift in the bulk copper peak and the existence of the voltammetric loop were evidence of a "preferred perpendicular orientation of the ligand". Furthermore, they invoke quantum-mechanical tunnelling barrier to explain the shift of the copper deposition peak, and suggest a type of autocatalysis to explain the voltammetric loop.

But all the above evidence is also consistent with a simple physical model in which Cu^{2+} ions are discharged directly onto the surface of the gold electrode. The electrode may be imagined as being, say, only 99.9% covered by the monolayer and not 100% as claimed. Such a coverage would be sufficient to eliminate all but 0.1% of both the copper 'underpotential' and Fe^{2+}/Fe^{3+} peaks, making them undetectable on the scale used, but would leave enough area of uncovered gold to allow the nucleation and growth of bulk copper crystals. Moreover, the high coverage would also block a large fraction of the active sites for nucleation on the gold surface, so that the nucleation overpotential for copper deposition would be increased by several hundred millivolts, precisely as observed. The loops in the voltammograms are no more than the expected response of a nucleation-and-growth process to a triangular scan of applied potential, as proved elsewhere[2].

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RUBINSTEIN *ET AL.* REPLY—Fletcher challenges our main conclusion, that the selective response of the gold electrode/(TBEA+blocking component) system to Cu^{2+} ions in the presence of, for example, Fe^{3+} is due to selective complexation of Cu^{2+} ions by TBEA molecules, and suggests an alternative interpretation. But experimental evidence[1] and other data clearly support our selective complexation model and exclude Fletcher's suggestion.

Selective behaviour of the same kind as we reported is observed when TBEA is replaced with TBPA or when the blocking agent OM is replaced with naphthol polymer (NP) (Fig. 2 in ref. 1) or polymerizable octadecyl trichlorosilane (OTS)[3,4] (*a* in the figure). As we have shown previously[3,4], diffusion-controlled faradaic currents are generally enhanced at randomly distributed pinholes. A situation in which the bulk-copper peaks are prominent whereas copper 'under potential' and Fe^{3+}/Fe^{2+} peaks are totally absent would therefore require rather unusual size and spatial distributions of pinholes. It is unlikely that three different blocking elements and two different ligands lead to the same special kind of pinhole distribution. Moreover, being deposited electrochemically, NP should block all uncoated electrochemically accessible sites. An NP layer of comparable thickness on a bare gold surface indeed blocks copper deposition completely[1].

Ionic competition experiments provide strong experimental support for the selective complexation model. Zn^{2+} and Cu^{2+} ions, which resemble Cu^{2+} in coordination requirements, successfully replace Cu^{2+} at monolayer ligand sites although being electrochemically inactive under the present conditions. The Cu^{2+} peaks are suppressed in the presence of Zn^{2+} (*a–c* in the figure), and at a high Zn^{2+} concentration (~ 8.0 mM) the Cu^{2+} peaks cannot be detected at all. The same is observed with Cu^{2+} (in both cases the peaks are fully recovered in pure Cu^{2+} solution). Conversely, the presence of non-complexing ions, such as Fe^{3+} or Ce^{3+}, has essentially no effect on copper deposition (Fig. 2 of ref. 1; *d* in the figure). These results are consistent with complexation selectivity, but not with reaction at uncoated areas. Moreover, the complete disappearance of Cu^{2+} peaks in these competition experiments indicates the absence of electrochemically active pinholes down to <0.005% of the total area.

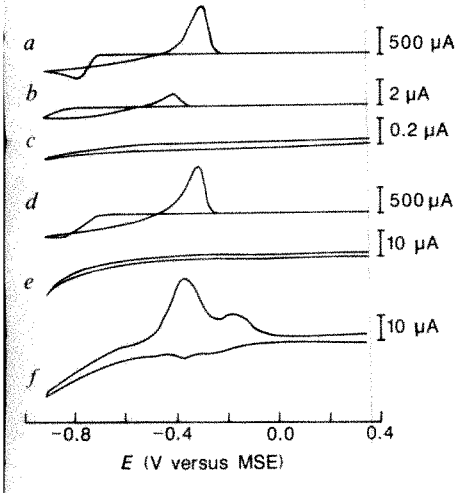
Fletcher suggests that the active element (TBEA or TBPA) plays no role in the observed selectivity. Figure 1*e* and *f* shows control experiments with pure OM monolayers on gold, in which a small number of pinholes were deliberately introduced (manuscript in preparation). At ~ 99.9% electrode coverage[4], Fe^{3+}/Fe^{2+} peaks indeed disappear whereas Cu^{2+} peaks still persist. However, these peaks (< 4% of the intensity observed with gold/(TBEA+OM or OTS)) clearly indicate normal copper deposition on bare gold (Fig. 2*a* of ref. 1), in that they show prominent copper 'underpotential' features and no unusual overpotential for bulk copper nucleation (as Fletcher suggests will be the case for pinholes). The presence of Zn^{2+} in solution here has, as expected, no effect on copper deposition. Thus, the characteristic features of Cu^{2+} reduction cannot be observed in the absence of the active component.

Fletcher's suggestion of a nucleation overpotential rather than a tunnelling barrier does not contradict our basic model. The overpotential and voltammetric loop, indicative of a barrier to electron transfer which diminishes as metal deposition starts, can be caused by either or both of these phenomena; its precise nature has no bearing on our principal conclusions. We suggested the tunnelling barrier as a possibility based on the observation of an increased barrier when using TBPA in place of TBEA, and consequently an increased Cu^{2+}-to-electrode distance. The thickness of a TBEA monolayer — 9.5±1.0 Å as measured by ellipsometry — supports the assumed perpendicular orientation of the ligand molecules, and thus the assumed distance of complexed Cu^{2+} from the electrode. Furthermore, the overpotential and loop cannot be reproduced in the absence of TBEA or TBPA.

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Cyclic voltammograms in 0.1 M H_2SO_4 containing different ions (scan rate =0.10 V. s^{-1}; electrode area =0.63 cm^2; MSE is mercurous sulphate reference electrode, +0.400 V versus saurated calomel electrode). *a*, Au/(TBEA+OTS) in 1.0 mM Cu^{2+}; *b*, Au/(TBEA+OTS) in 1.0 mM Cu^{2+} + 6.0 mM Zn^{2+}; *c*, Au/(TBEA+OTS) in 1.0 mM Cu^{2+} + 8.0 mM Zn^{2+}; *d*, Au/(TBEA+OTS) in 1.0 mM Cu^{2+} + 6.0 mM Ce^{3+}; *e*, Au/(OM with controlled pinholes) in 3.0 mM Fe^{3+}; *f*, same as *e* in 1.0 mM Cu^{2+}.

Rapid reversibility of lake acidification?

SIR—Battarbee *et al.* recently claimed[1] that diatom cores showed "a trend towards progressively decreasing acidity" in two Scottish lochs. They put these data forward as evidence for rapid reversibility of acidification in Galloway lochs in response to a decrease in acid deposition, in contrast to model predictions which show a much slower response. Unfortunately, the data do not justify this optimistic conclusion, although they do raise some interesting questions.

Battarbee *et al.*[1] do not present the customary diagram of *p*H change with time on the grounds that the changes observed are within the errors of the model for *p*H reconstruction. The diagram can be constructed (Fig. 1) from measurements on their Fig. 2 (which incidentally contains a mistake: the percentage frequency figures would be multiplied by 2) using the index B method described elsewhere[2]. There is clearly no simple relationship between the inferred *p*H of Loch Enoch and sulphur deposition at Askalemuir nearby[3], or between inferred *p*H and SO_2 emissions in the United Kingdom. A reasonable inference would seem to be that the *p*H of Loch Enoch is not sensitive to short-term (10-yr) changes in sulphur emissions of up to 40%, within the limitations of the diatom core method (±0.3 *p*H units[2]).

To reduce the uncertainty of the diatom methods, Battarbee *et al.* used correspondence analysis, an ordination technique which aims to simplify the structure of multivariate data by projecting it onto several principal axes[4]. The difficulty with such techniques is that the axes have no direct physical integration: this has to be inferred from other evidence. One criterion for a successful ordination is tht replicate samples show the same patterns[4]. The two-dimensional projections of Batterbee *et al.*[1] show similar patterns only in that the general direction for replicate cores in each loch is the same. However, in one loch the time track goes east–west. Recent changes in direction are meant to indicate recovery, but in Loch Enoch the time track which had been proceeding south–east turns west and then north, whereas in Round Loch of Glenhead the 1984 core track describes a little circle and turns north–west, and the 1986 core which was going west–north–west turns south–east.

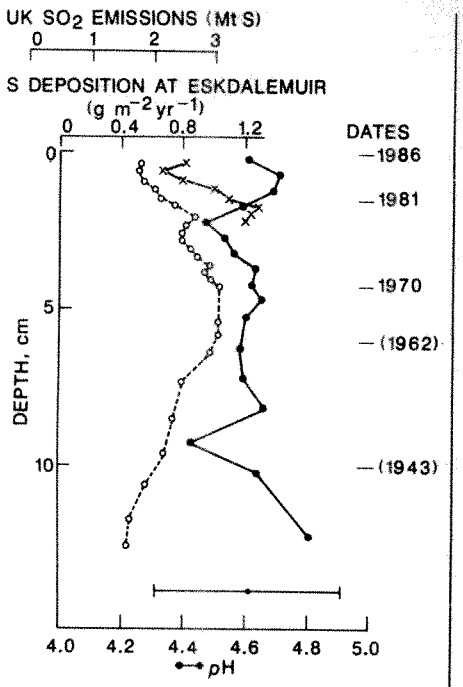


Fig. 1 Inferred *p*H (index B)[2] in Loch Enoch from Fig. 2 of ref. 1 (closed circles). Dates are taken from ref. 1 and, by inference, from the 1982 core (in parentheses). The estimated standard error of the method[2] is shown below. Sulphur deposition at Eskdalemuir[3] 80 km distant (crosses) and UK SO_2 emissions[3] (open circles) are also shown.

It is difficult to see how such diverse behaviour implies acidification followed by recovery, particularly as the three tracks in Fig. 4 of ref. 1 occupy different positions in two-dimensional space.

The chemical evidence presented by Batterbee *et al.*[1] appears more compelling, particularly as the 'non-marine' sulphate has decreased roughly in parallel with deposition, implying that these catchments do not store or release sulphur. However, only two or three samples per year were taken and there have clearly been influences on water chemistry other than sulphur deposition. The 45–60 per cent increase in Cl^- between the 1978 and 1984 samples shows there were one or more sea-salt episodes influencing the chemistry of the later period. The non-marine calcium has doubled in one loch, a result completely at variance with correct understanding of acidification and de-acidification processes[5], which would predict a decrease in Ca^{2+} with reducing SO_4^{2-}. Has there been some increase in calcium input to these catchments or some catchment disturbance (which would also account for the increase in sedimentation rate[1])? I conclude from the paper[1] that diatom-core data have no rapid recovery resulting from reduction in SO_4^{2-} deposition, but that direct chemical data show intriguing patterns for further investigation.

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BATTARBEE *ET AL.* REPLY—Skeffington's attempt to dismiss our evidence for the reversibility of lake acidification seems to be based on a misreading or misunderstanding of our paper[1]. First, we do not argue for a rapid recovery, rather for "little delay ... in the response.... to a decrease in acid deposition". The extent of the recovery is quite minor.

Second, we argue that the sensitivity of diatom–*p*H reconstruction models is too low to be of value in this context where the change is about 0.1–0.2 *p*H units, yet Skeffington presents such a reconstruction.

Third, we argue for floristic reversal as a more sensitive indicator and the ordinations presented clearly show that present diatom assemblages are similar to pre-1970 floras. It is not necessary to show that the axis is a *p*H one as the acidification histories of both these sites are already known[6,7]. To demonstrate this point, we use in Fig. 2 a canonical correspondence analysis[8] of the Loch Enoch data as before, but here constrained by the modern chemistry and diatom assemblages of other Galloway lakes. The first axis is clearly identified as a *p*H-related axis.

Fourth, Skeffington has misread the frequency of chemical sampling. Samples were taken at 2–3-month intervals, not 2–3 times per year.

Fifth, increases in sea-salt inputs reduce the *p*H of surface waters. Therefore, contrary to Skeffington's implied argument that the higher sea-salt concentrations in the 1984–85 data set would reduce the *p*H difference between the sampling periods, the differences would have been greater if the 1978 values had been maintained.

Sixth, it is not relevant to use the usual sea-salt ratios to calculate non-marine calcium because cation exchange processes can also produce elevated calcium levels as well as lower *p*H values in surface waters with sodium being retained on soil-exchange sites[9].

Contrary to Skeffington's view, both the floristic and chemical data presented in our paper[1] can only be interpreted as evidence for reversibility. It would be useful to assess whether similar responses have occurred at other sites with differing catchment characteristics, and to consider the extent to which reversals can be sustained if sulphate deposition were not further reduced[10].

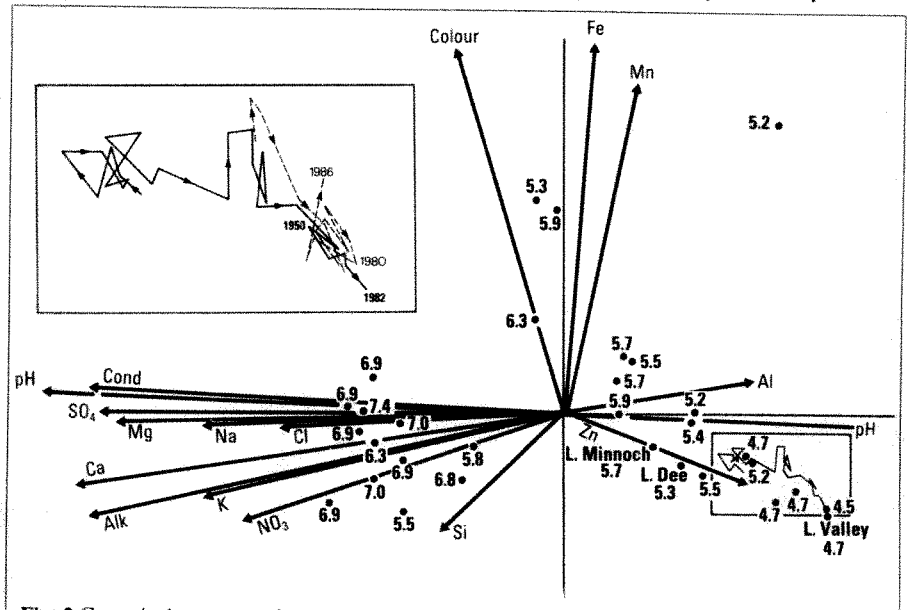


Fig. 2 Canonical correspondence analysis of Galloway lakes showing environmental axes and the *p*H of sites. The time-track for the 1982 Loch Enoch core is also shown. The inset shows an enlargement of this time-track along with the time-track for the 1986 core.

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Whale correction

IN the letter[1] by K. Ralls and R. Brownell Jr, refs 4–6 were omitted from the reference list. They are reproduced below.

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The inquiry inquiry

Wolfgang Rüdig

Sizewell B: An Anatomy of the Inquiry. By Timothy O'Riordan, Ray Kemp and Michael Purdue. *Macmillan, London: 1988. Pp.474. £45.*

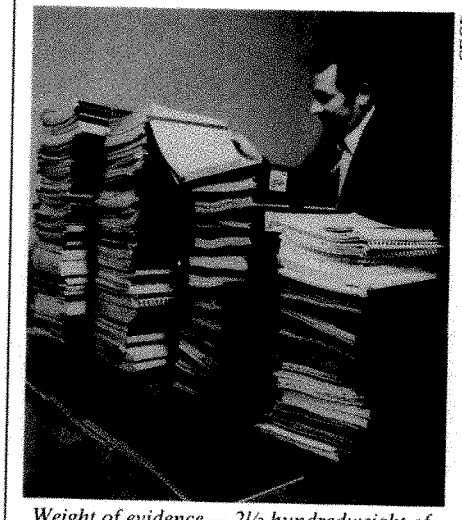
EVER since the Windscale inquiry of 1977, the public inquiry has been central to any discussion of the politics of nuclear power in Britain. The 100 days of Windscale made the 'civil' use of nuclear power a prominent issue in British politics, and debate over the virtues and drawbacks of the 'ritual' of nuclear inquiries produced countless articles and books. When in 1980 the government announced its intention to hold a public inquiry into the first pressurized water reactor (PWR) to be built in Britain, the stage was set for further argument about public involvement in reaching decisions about nuclear power.

The inquiry into the PWR project at Sizewell in East Suffolk eventually opened in July 1982. It broke all records in terms of its length and costs, finally being closed in March 1985 after sitting for 340 days. The inspector's report was formally submitted to the responsible minister in December 1986; his consent for the project to go ahead was given in March 1987. Work started in the same month, more than seven years after the respective nuclear-expansion programme had first been announced by the government. But by then, there was no powerful opposition movement making a determined effort to stop it. Although the political fall-out of Chernobyl had swept away most other nuclear development programmes in Western Europe, Britain was able to start a new phase of nuclear expansion without any serious difficulties over legitimation. Any analysis of this situation has to take the Sizewell B inquiry into account.

A team from the University of East Anglia, led by Professor Timothy O'Riordan, was given the task by the Economic and Social Research Council of analysing the inquiry. The results of the project have now been published. Mirroring its subject matter, *Sizewell B: An Anatomy of the Inquiry* can claim to be the longest and costliest book ever written on a British public inquiry. After five years of research, which was completed in May 1987 and has resulted in this well-produced book, we may ask "Was it worth it?". Do we now know more about the Sizewell B inquiry, the reasons for its length and costliness, its result and the political repercussions? Are we now better placed to evaluate the political conflict over nuclear energy and assess the effect of the inquiry on nuclear policy making?

The authors certainly faced a mammoth task in coming to grips with the unprecedented amount of material generated by the inquiry. They help the reader by including a number of appendices providing, *inter alia*, information on the chief parties involved and an indispensable list of acronyms (a total of 159). Armed with such auxiliary implements, one can delve into the maze of 12 chapters, covering a further 413 pages, which examine the inquiry from different angles.

Was it worth it? On the positive side, the description of the events surrounding a number of key issues is a notable



CEGB

Weight of evidence — 2½ hundredweight of it at the Sizewell inquiry from the Central Electricity Generating Board alone.

achievement. The arduous routine of attending endless inquiry sessions and meetings, and working through the heaps of papers involved, deserves credit which should go particularly to the senior research associate, Dr Ray Kemp. On the analytical front, it is the treatment of the 'internal' features of the inquiry, such as the innovative introduction of an inquiry counsel, the 'top table', and the 'side-room meetings', which is worked out especially convincingly. Another strong point of the book is the coverage of the legal background.

The aims of the authors, however, go far beyond the provision of a descriptive account of the inquiry with limited examination of its legal aspects and the roles of the individual actors. They set out to give an authoritative account and a comprehensive analysis, explaining the course and the result of the inquiry, and to provide recommendations for the future. It is measured against these more ambitious claims that the book must be considered a failure.

No integrated theoretical framework is developed to analyse the inquiry. Some individual snippets of social science theory are alluded to, but reference to them is patchy and marginal. The questions raised by these theoretical points are pursued separately, so much of the analysis remains rather disjointed. We find a normative–legalistic evaluation of criteria of fullness, fairness and thoroughness going hand-in-hand with attempts to see the inquiry as a means to gain legitimation for a predetermined decision. Both approaches also have to compete against an implicit policy orientation, which is never developed theoretically, analysing the inquiry essentially as an adminstrative tool.

As a result, there are some glaring contradictions. For example, the reader is told fairly early in the book that the outcome of the inquiry was "inevitable" because the "technology, its supporting organizations and the people involved were just too powerful, too big and too committed to be deflected" (p.39). One chapter later, however, we learn that the public inquiry cannot just be regarded as a place for objectors to let off steam, but that it is an "extra-parliamentary device for political debate where conflicts of interest over principles and priorities of natural import can democratically be played out" (pp.52–53). No explanation is furnished of the "democratic" nature of such an exercise. Further, there is a range of suggestions for future public inquiries with the aim of making them, amongst other things, more "efficient". In the light of the previous thesis on the "inevitability" of the outcome, one is bound to ask who would benefit from more "efficient" ways of conducting such inquiries.

O'Riordan and his colleagues see the main result of the Sizewell B inquiry as being its lasting effect on the management of nuclear projects and the conduct of the licensing process. This aspect of the inquiry's ramifications is well worked out and convincingly argued. But, apart from rationalization of the nuclear sector's own procedures, has the inquiry managed to gain legitimacy for a nuclear project whose inevitability the authors consider to have been established before and outside the inquiry? On this point, the book is very weak. While it is said that the factors determining the outcome of the inquiry are to be found outside the inquiry itself, no effort was made to investigate them more fully. Scant attention is given to the state of the nuclear industry and the anti-nuclear movement. Although the authors claim to have conducted intensive interviews with "key participants and observers" (p. viii) there is no further reference to these interviews in the book, and no

clear-cut judgement of the inquiry's influence on the future of the nuclear industry and the anti-nuclear movement in Britain. And although there is some assessment of the possible long-term implications of changes to nuclear project management, a discussion of the future public acceptability of nuclear power is absent.

Was the fullness and thoroughness of the inquiry effective in diverting opposition groups from potentially more effective means of protest? What happened to the groups that boycotted the inquiry because of its alleged unfairness? How can we explain the combination of a perceived unfairness, both in terms of the imbalance of resources between proponents and objectors, and the alleged predetermination of the outcome, with the apparent lack of public response to its conclusion? These questions are not seriously addressed. The book provides a thorough and authoritative account of the inquiry proceedings, but the final evaluation of the effects of the Sizewell B inquiry has yet to be made. □

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Immune response

J.R. Batchelor

Milestones in Immunology: A Historical Exploration. Compiled by Debra Jan Bibel. *Sci Tech Publishers, 701 Ridge Street, Madison, Wisconsin: 1988. Pp.330. $35. Distributed outside North America by Springer-Verlag, DM64, £21.*

COMPILING an anthology of scientific papers judged to be milestones in a subject can be a stimulating occupation on a winter's night, particularly in company with argumentative friends. Of course there will be areas of agreement as to what should be included and who made a particular observation, but strong disagreements are also likely to be voiced. Debra Bibel's *Milestones in Immunology* is therefore a provocative as well as an informative book.

To our shame, few of us read original articles published more than five or ten years before we started work on our own doctoral theses, particularly if they are not concerned with the limited fields in which we are engaged. This failing accounts for much of the dull incoherence and the narrow perspectives to be found in the introductory historical chapters of those same theses. Dr Bibel's book provides a partial solution. She has selected her 'milestone' papers, grouped them loosely into nine sections (immunotherapy, immunochemistry, cells and interactions, and so on), provided long excerpts of the papers, and accompanied them with commentaries on both the science and authors.

There is much to enjoy here, according to individual tastes. For example, I found it interesting to be reminded of the extraordinary bureaucratic resistance to the introduction of prophylactic immunization against typhoid for British soldiers at the beginning of the twentieth century, a measure advocated with much spirit by Almroth Wright. There is the concise logic of Linus Pauling's paper on the structure of antibodies in which he assumes, with Marrack, Heidelberg and others, that because antibody can form a precipitate on combining with antigen, the antibody molecule must have two combining sites. "The rule of parsimony (the use of the minimum effort to achieve the result) suggests that there are only two such regions, that is, that the antibody molecules are at the most bivalent". The fact that there is an exception to this rule, and that the 'instructive' theory of antibody formation he proposed in the paper is wrong, does not detract from the interest. There are, too, some apt or ironical quotations introducing the chapters. For example, the line from Edmund Burke ("Our antagonist is our helper") or that from Paul Anderson ("I have yet to see any problem, however complicated, which when you looked at it in the right way, did not become still more complicated").

In spite of many attractive features of the book, there are aspects which I found disappointing. To a large extent they are inherent in treating a scientific enterprise as if it consisted of a series of pedestrian studies, too dull to be noteworthy, punctuated by the inspired contributions of a few outstanding people.

Dr Bibel will not escape the charges of oversimplification and, at times, even injustice. To select one of G.D. Snell's papers describing a strategy for identifying the alleles of minor histocompatibility system genes in a series of congenic mouse strains, and use this as a reason for placing his work in the section on technology, is admitted by the author as likely to invite criticism; but it mistakes a branch for the whole tree and those who do not already know of the studies of Snell and his colleagues would not, from this book, be able to appreciate their theoretical significance. In my view, the importance of C.C. Little's hypothesis, formulated early this century — that progressive growth of transplanted tumours depended upon graft and host sharing multiple, independently segregating genes — should have been recognized. The hypothesis provided the motive for the development of inbred strains, which were needed to test Little's theory. Snell's great achievement was that he placed these genes into separate linkage groups, determined the extent of their polymorphism and defined their relative influence upon graft survival. This achievement led to the concept of each species having a single major histocompatibility system and multiple minor systems, as well as providing many invaluable mouse strains as tools for research.

In the description of Peter Gorer's work, Dr Bibel comments briefly:

> His new finding, however, was the correlation of resistance to tumor transplantation to the absence of a particular blood group antigen. He offered tumor studies an immunological perspective, and provided the first linked marker of histocompatibility.

Few would guess from this remark how clearly Gorer's experiments had shown that the fate of a transplanted tumour depended on whether or not the recipient mice mounted an immune response against antigen II expressed by the tumour. And it seems strange that after Gorer had thus demonstrated the immunological basis of graft rejection, Dr Bibel does not cite his pithy conclusions:

> Normal and neoplastic cells contain isoantigenic factors which are genetically determined. Isoantigenic factors present in the grafted tissue and absent in the host are capable of eliciting a response which results in the destruction of the graft. Under special circumstances the response may not be elicited or grafted tissues may not be destroyed thereby.

I also miss acknowledgement of the work of so many scientists who deserved to be mentioned. For example, in the commentary on Jean Dausset's discovery of human leukocyte alloantibodies, and the analysis of the HLA system carried out at the international workshops, there is no recognition of how the success of these analyses depended critically on the solution of at least three problems. First, van Rood showed how to recognize antisera containing antibodies of the same specificity, using computers to perform the large number of χ^2 tests required. Second, Walford and colleagues discovered that rabbit complement allowed Gorer's lymphocytotoxicity test to be used with human reagents, thus providing a reliable test. Third, Terasaki and co-workers invented the 'micro' version, thus permitting small amounts of defined antibody reagents to be used by many laboratories for standardization purposes. None of these was a small achievement. Each is an example of how misconceived it is to imagine that progress in understanding depends upon the efforts of a few; the reality is that it is due to the applied talents of the many. □

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Life and loves of a Martian pioneer

Peter Kemp

Genesis: An Epic Poem. By Frederick Turner. *Saybrook, 4223 Cole Avenue, Dallas, Texas 75205: 1988. Pp.303. Distributed by W.W. Norton, pbk $9.95.*

INSPIRED by such varied sources as Homer, James Lovelock's Gaia hypothesis, the Bible and the Wisconsin Arboretum's success in turning degraded farmland into prairie, Frederick Turner's *Genesis* is a work of considerable compass. Aiming to synthesize old and new, art and science, it tells in epic verse the story of the colonizing of Mars: as the settlers contrive to transform an arid planet into garden, Turner seeks to re-fertilize an apparently defunct genre.

His task is as risky as it is ambitious and, conscious of this, he equips his epic with defence mechanisms. This 10,000-line saga is, we are informed, the work of a twenty-first-century poet living under mild-ish totalitarian conditions then prevailing on Earth. A martyr to arthritis and despondency, he is painfully aware that his verse lacks polish. Also, we are warned that in the process of projecting it into Turner's cortex — exactly how is not explained — clumsiness may have crept in. Further intimating that the poem should be handled with kid gloves is a heartfelt evocation — "Poor science-fiction. Last muse of the gods . . ." — lamenting the unjustly despised status of this type of writing. As a final deterrent against criticism, the poem features as its most repulsive character a reviewer who has "A Shiite mullah's openmindedness, /A moral backbone of boiled watercress;/All the prophetic vision of a sheep".

At risk of seeming such a mix of bigotry, salad and mutton, it has to be said that the poem is uneven. Its best stretches are its landscapes. Turner sensuously excels at evoking atmospheric terrains — from the wind-scorched Australian outback to Mars at different stages of its ecogenesis: the "golden furs and powdery crimsons" of mosses and lichens spreading over "glitterings of slime and foams", blossoms gorgeously exploding into newly oxygenated air.

But, along with this flair for dramatizing the processes of life-formation and evolution, goes an enfeebling inability to create and develop lifelike characters and situations. The poem is peopled by melodramatic puppets. Resembling the plastic personnel of some *Star Wars*, they range from evil Gaea, perfidious leader of the Ecotheist Movement, which teaches that nature must never be interfered with, to noble Chance, her estranged husband, dedicated to bringing life to Mars. As battle-ships, a space Ark and a displaced ice moon of Saturn hurtle round the Solar System, freedom fighters, visionaries, gladiators, lurid villains and a miracle-working prophetess engage in comic-strip adventures.

Bizarrely, Turner's celebration of advanced biotechnology is studded with throwback violence: hand-to-hand fights, karate chops, disembowelling. Martial arts loom as large as Martian pioneering in his story. Incongruity keeps breaking through the attempt to combine sophisticated scientific lore with the barbarities traditional to epic literature: wars, parricide, split families, blood feuds, vengeance.

The poem's classic *in medias res* opening finds an appropriate out-of-this-world parallel to the *Iliad*: helmeted figures in metallic uniforms clash on the deadly plains of Mars. But despite the subsequent efforts to elevate proceedings by using the apparatus of the epic — heroic similes, addresses to the muse, cataloguings of troops — bathos often intrudes. After an assassin returns from a mission to kill the Sibyl at her shrine in the Peacock mountain on Mars, it comes as an anti-climax to hear of his booking into a "clean hotel just west of Reading". Behaviour oscillates between the cosmically marvellous and the comically mundane. The switches of language also jar, with abrupt changes from laboratory terminology ("volatile calyptral ribosomes", "synthesized endorphins", "heterodyning frequencies") to musty archaisms ("And ruddy Phoebus gins to welke in west"). And it seems a shame that the twenty-first-century poet, though familiar with the intricate mechanisms of planetary engineering, has not always worked out ("tech-/Nical", "psych-/Ologically", "choc-/Olate") how to fit his words neatly into pentameter lines. □

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We've got rhythm

Asa Briggs

The Metronomic Society: Natural Rhythms and Human Timetables. By Michael Young. *Thames & Hudson/Harvard University Press: 1988. Pp. 301. £16.95, $25.*

MICHAEL Young, whose book *The Rise of the Meritocracy* fascinated readers a generation ago, has produced another remarkable and equally stimulating book which deals with even more basic issues. It is designed to be read by a wide audience, and it abounds in memorable phrases and descriptions. "Durability is not durability unless it is pulsating." "Habits are not usually chosen with any deliberation; they just grow, wild flowers rather than cultivated ones." "A habit is a memory unconsciously edited for action." "The fertility of nurture festoons itself around nature." "Time is immanent in everything . . . . You cannot catch it because you already have it." In the broadest sense this is a book for everybody, rich both in analogies and in insights. It deals in various degrees of detail with such topics as time, memory, tradition, habit and custom. It begins with an analysis, continues with a set of explanations and ends with a prescription.

The term "metronomic society" is not introduced until the end of the first chapter, where we are told that "modern society (and social science with it) . . . has a linear bias to it, and that with this linear bias many natural rhythms have been replaced by artificial ones, a rhythmic society replaced by a metronomic". The second chapter, which begins by noting how the living organism has long been one of the favourite analogies for society, examines more fully the nature of the natural rhythms. It summarizes research, not all of it completely convincing, on an internal biological clock, while adding that it will not be conclusive until the clock or clocks shall be found.

Lord Young then turns to social behaviour and how it is timed, drawing the necessary contrasts both between natural rhythms and artificial rhythms and between animals and human beings: "bees and birds do not celebrate Sundays". Regularities, both natural and artificial, "create the social mesh", but "particularly within large and complex organisations, biological and social rhythms are in conflict with each other". The fourth chapter, "Habit: the Flywheel of Society", is the most original, although the term habit is used very generally to cover phenomena which are only differentiated at the end of the chapter (via the *Encyclopedia of Social Science*) into motor habits, cognitive habits, emotional habits and moral habits. It would have been more persuasive if the differentiation had been introduced from the outset, for the various activities that Lord Young uses as examples of habit suggest differences more than similarities. The conclusions are firm, however — "Habit is a means of making learning stick"; "Habits constitute the rules of individual behaviour and customs the rules of social behaviour".

There is a darwinian ring to the last paragraph of the fourth chapter, which sets the scene for the following discussion of "Social Evolution". This is a less original chapter. It deals with speech, law and institutions, taking the British monarchy as the example, and notes how "despite and because of the hold of habit, institutions can evolve" and how in each

case "change comes not by a wholesale overthrowing of the past but by leaning against the past to gain a kind of leverage and add something new to it". A glance at another Young, G.M. Young, might have been useful here. Even so, Lord Young, who draws most of his analogies from biology, has some useful comments to make about history. In comparison economics throughout gets short shrift, even in the brief section "The Question of Progress", and even though economists have been particularly interested, as the author observes *en passant* earlier in the book, in cycles and in 'trends' (a term he does not use).

Nonetheless, Chapter 6, "The Metronomic Pulse", begins with economics although it is approached ritualistically rather than analytically, and later turns to a point made by a number of economists that "the axiomatic and automatic consequence of a rising standard of living is that time becomes scarcer". For Lord Young the position is more serious than they suggest, for "the symptoms of the famine are all around us" and (a memorable phrase) "the more deliberation there is, the more deliberation is necessary". The thought leads, in linear fashion, to the prescriptions of the last chapter — reintroducing "more of the cyclical into the evolutionary mix" and giving "a more prominent place once again to the cycles of nature". The penultimate section is called "Back to Circadian Rhythms", the last "Escape from the Grid". The titles speak for themselves. To anyone suffering from time famine the prescription is attractive, and there may be reasons different from those advanced by Lord Young for following it. He does not quite say, however, how to do so.

Within the book there are running arguments with sociologists about the nature of sociology, at least one dig at anthropologists, and freely acknowledged processes of borrowing from biologists. Indeed, while expressing distaste for sociobiology Lord Young shows himself to be an eloquent spokesman for his own version of social darwinism. He has great social concern, however, and is untouched by determinism. One word he never uses about human beings is curiosity — perhaps because he has so much of it himself.

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New in paperback

- *Medicine in China* by P.U. Unschuld. Publisher is University of California Press, price is $12.95, £7. For review see *Nature* **327**, 198 (1987).
- *The Great Devonian Controversy* by Martin J. S. Rudwick. Publisher is University of Chicago Press, price is $22.95, £15.95. For review see *Nature* **316**, 491 (1985).
- *Duchenne Muscular Dystrophy* (revised edition) by A. E. H. Emery. Publisher is Oxford University Press, price is £17.50, $35. For review see *Nature* **329**, 594 (1987).

Infant individuals

George Butterworth

Nature and Nurture During Infancy and Early Childhood. By Robert Plomin, John C. DeFries and David W. Fulker. *Cambridge University Press: 1988. Pp 345. £30, $44.50.*

In this book, Robert Plomin and his colleagues employ quantitative behavioural genetics to explore the origins and development of individuality during infancy and early childhood. They analyse "the nature of nurture" to study genotype–environment interaction and correlation, and the genetic mediation of environmental influences, rightly stressing that their methods yield totally different information to that obtained by research on group averages. The second approach consigns individual differences to error variance, whereas the first respects natural variation as being at the very foundations of individual development.

Behaviour genetics involves detailed and rather technical statistical analyses of twin and adoption studies. Twin studies, in which identical and fraternal twins are contrasted, enable estimates of the components of variability due to genetic or environmental effects. Adoption studies, on the other hand, allow the possible covariation between genes and environment to be teased apart. Genetically unrelated individuals adopted into the same family will resemble each other for environmental reasons only. The effects of the remainder of the non-shared environmental variance, such as differential maternal attention between siblings, can also be estimated.

Height and weight, obvious physical variables, are often used to 'anchor' cognitive and behavioural measures. Height, for example, shows a strong correlation from infancy to early childhood, with genetic and environmental effects differing at various times in development. Fraternal twins are actually physically more alike than identical twins at birth, but not at six months, perhaps because identical twins may be subject to greater intra-uterine competition for nourishment through the chorion. Adopted siblings also show significant correlations for height during the second year of life, although these values are not as high as for fraternal twins. Hence, for physical variables it is possible to distinguish genetic effects, effects of the shared twin environment and effects of non-shared environmental factors.

Cognitive or personality development is, of course, more variable than physical development. IQ scores are notoriously unstable from infancy to early childhood, at least in part because of the different sensori-motor and verbal items on the tests. Genetic influences on IQ may be greatest between the ages of one and two years, while shared environmental factors, as assessed through adoptive siblings, may account for more of the variability between IQ in early childhood. The Colorado Adoption Project (CAP) analysed here also shows that shared environment may be of lesser importance for the development of temperamental characteristics than non-shared environmental effects. An odd, unexplained result of the CAP study was that the amount of television viewing at three and four years of age may have a heritable component; a significant correlation was found on this variable between adoptive siblings.

In the final chapter, the authors consider the "nature of nurture". The appropriate behavioural genetic design for such a study would involve quantifying the parenting behaviour of adult identical and fraternal twins as well as data from twin children and their parents. Genetically mediated differences in parenting, as revealed by differences between identical and fraternal twin parents, may have differential effects on their children. Genetic differences among children may also contribute, and the differential effects could be quantified because the children of identical twins are related to each other as half-siblings, whereas the children of fraternal twins are cousins. Parents and children share 50 per cent of their genes, regardless of whether their twin children are identical or fraternal, and this again yields the possibility of contrasting differential effects on development. Unfortunately, there are no data on these contrasts; in the book they seem to be mentioned as a behaviour geneticist's methodological ideal. But adoption studies do offer some evidence that maternal involvement — in encouraging development, for example — correlates more highly with IQ in non-adoptive siblings than in the adoptive child. The authors suggest that this may be explained as a genotype–environment correlation whereby the greater genetic match between the mother and her biological child facilitates development.

The book contains descriptions of many hundreds of observations on the complex inter-relationships between heredity and environment, and they illustrate well the ingenious ways in which the components of individual variation can be disentangled. The authors have made a great deal of progress beyond the stark genes versus environment dichotomy of earlier behaviour genetics. Whether the greater sophistication of partitioning will satisfy those who espouse a holistic approach to individual development remains to be seen; but at least the inter-penetration of nature with nurture has been acknowledged. □

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Graduates — for better, for worse

J.M. Ashworth

Should a university be devoted solely to teaching? Data from the University of Salford suggest that such an institution would be unlikely to produce good graduates.

BRITISH universities receive grants from the state through the University Grants Committee (UGC) which they use to support both the education of undergraduates and the research of their staff. The British government has suggested that universities should account separately for what they spend on research and on teaching, which has given rise to a debate on the extent to which the quality of undergraduate teaching requires that those doing the teaching should also be carrying out research.

In a recent report[1], the Advisory Board for the Research Councils (ABRC) recommended that British universities should be classified into groups, with one group (type T) being defined as those that are unable to provide comprehensive facilities for research. The government has rejected this advice, but many fear that such a policy may be introduced by stealth as a result of the current UGC reviews of university departments of subjects such as physics and chemistry. The UGC will be replaced by the new University Funding Council (UFC) in April 1989, and it is this body that will have to decide whether or not to implement the recommendations of the reviews.

Lack of data

The debate has been marked by a lack of data. Those who believe that high-quality teaching can be carried out only in a university which is also a research centre have been placed at a disadvantage by the fact that, although it is relatively easy to adduce anecdotal evidence for the proposition that 'not all good researchers are good teachers' (or vice versa), it is not easy to demonstrate that good teaching depends on good research. It is the purpose of this article to provide such a demonstration.

In July 1981 the UGC informed the University of Salford that the quota of British students the university was expected to admit was to be reduced by 30 per cent, and that the recurrent grant that the university had been expecting to receive over the next three years would be reduced by some 44 per cent. This precipitated a crisis: the salaries of approximately a third of the staff could no longer be paid from UGC funds, and most of those concerned retired prematurely. Efforts were also made to obtain funds from other sources, and these have been relatively successful; the university's income is now larger in real terms than it was in 1981, more than half of it coming from sources other than the UGC recurrent grant. What is less well known are the consequences that this restructuring has had on teaching in the university.

Honours degrees awarded by British universities are graded into classes. One measure of the quality of a university is the fraction of its graduates who obtain first-class degrees. Another is the 'average' class of degree, calculated by assigning a value of 5 to a first-class degree; 4 to an upper second; 3 to a lower second; 2 to a third; and 1 to a pass degree and then dividing this sum by the total number of graduates. The first of these measures can be taken as an indication of the extent to which a university acknowledges the exceptionally good student, and the second the extent to which it caters for the average student. Figure 1 (see over) shows how these measures have varied at the University of Salford since 1980; both have shown considerable improvement, but the change in the 'average' class of degree is, statistically, the more significant. In the analysis that follows, I have used these measures of student achievement as indicators of teaching effectiveness; both give the same outcome.

The improvement shown in Fig. 1 is surprising, for the number of academic staff (Table 1, column 1) fell by slightly more in percentage terms than did the number of undergraduate students in the same period (Table 1, column 4). Moreover, this smaller staff was under great pressure to obtain research grant and contract income from non-UGC sources, in which they were very successful; the research income of the university and the income of the commercial company (Salford University Business Services Ltd), whose profits are covenanted to the university, have both increased considerably (Fig.2). But how can a smaller number of academic staff, doing more research and consultancy, nevertheless construct an environment in which graduates obtain better classes of degree?

Explanation

The first possible explanation is that the standard of the degrees awarded by the university declined after 1981. This cannot be completely ruled out, but it is highly improbable. All degrees at the university are moderated by external examiners; in any year, about 60 examiners drawn from a wide variety of British universities and industries are involved in our examining boards. A conspiracy to reduce standards steadily over a period of years on the part of so many different professional people (most of whom are strangers to one another) is difficult to imagine.

If the quality of the university's degrees has remained constant, explanations of the changes shown in Fig. 1 must be due either to an increase in the quality of the students admitted or to an improvement in the effectiveness with which those students are taught.

In Britain, the school-leaving examinations (A-levels) are graded A–E; all universities require a minimum of two E-grade passes in two subjects at A-level for admission. Different grades can be given numerical values (A=5, B=4, C=3, D=2, E=1) and thus an 'average' A-level grade of the population of students admitted can be determined. In Fig. 3 the average A-level grade obtained by the entering students is plotted as a function of the year of

Table 1 Changes in the numbers of undergraduate students and graduates (of all kinds) at the University of Salford, 1980–1 to 1987–8

Academic year	1 Full-time UGC-funded staff	2 Full-time graduate students	3 Departmental research assistants	4 Full-time undergraduates	5 Total graduates*	Under-graduates/ graduates†
1980–1	469	336	130	4,205	935	4.5
81–2	467	286	123	4,014	876	4.6
82–3	387	288	158	3,731	833	4.5
83–4	348	362	124	3,342	834	4.0
84–5	328	423	169	3,154	920	3.4
85–6	346	517	184	3,104	1,047	3.0
86–7	344	498	201	3,151	1,043	3.0
87–8	335	580	197	3,245	1,112	2.9

* Sum of columns 1,2 and 3. † Column 4/column 5.

admission. Comparison of Fig. 1 with Fig. 3 shows that there is an approximately three-year lag between the rise in the A-level grades of the students admitted and the rise in the standard of degrees awarded. Because most undergraduate degree courses at Salford last three or four years, this suggests that the increased quality of the output is a result of the increased quality of the input. But closer examination reveals that this cannot account for more than a small fraction of the change. First, a sizeable fraction of Salford students (15–24 per cent, depending on the year) have entering qualifications other than A-levels (usually Business and Technician Education Council awards from colleges of further education). Such students obtain first-class degrees in at least the same proportion as their representation amongst the graduating class.

Second, the correlation between the class of degree awarded to a student and the A-level grades that that student had on entry three or four years previously is as poor at Salford during the 1980s as it has been previously[2], or as it is reported to be by other institutions[3]. So although the increased academic attainments of most of those admitted to the university undoubtedly contribute to the increase in the quality of the output, the relationship is not simple, and neither can it account for more than a fraction of the changes shown in Fig. 1.

At least as important seems to be the nature of the environment in which the students are taught. In 1982, as part of the process of adaptation that the university had to make to the loss of so much of its UGC support, the senate adopted specific and quite detailed "Aims and Objectives". The section in this document headed "Teaching" states:

The university's teaching is intended to result in graduates . . . with certain . . . characteristics:

(a) the capacity to acquire, organise and systematise knowledge and thereby to develop what the Robbins Committee called 'the general powers of the mind';

(b) the capacity to appreciate, to value and to make judgments of what is beautiful, of good repute and fit for its purpose. This involves the education and training of feelings and emotions as well as implying a moral or ethical framework within which (a) above must be attempted;

(c) the capacity to identify, formulate and then to solve problems and to make, design, organise, produce or construct useful objects and services;

(d) the capacity to co-operate with others, to value communal endeavour and achievement as well as competition.

Although the first two of these objectives are common to most universities, at Salford we believe that the other two are distinctive and that they encapsulate the university's purpose to 'educate for capability'.

This philosophy has helped to shift the

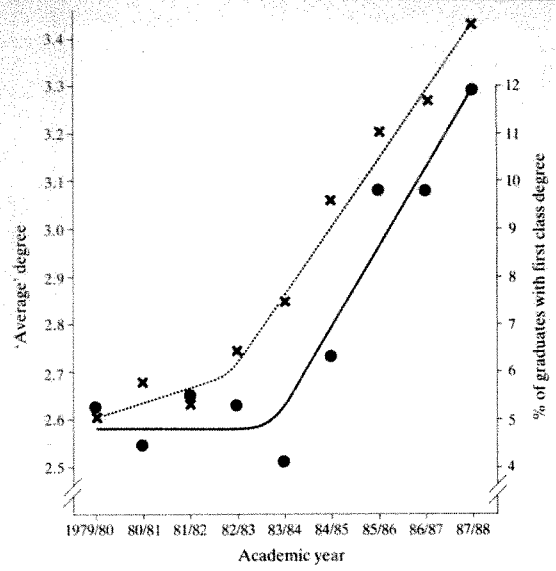


Fig. 1 Degrees awarded by the University of Salford between 1980 and 1988. The 'average' class of degree (crosses) is calculated by assigning a value of 5 to a first-class degree down to 1 for a pass degree, and then dividing the sum by the total number of graduates. The percentage of graduates obtaining a first is plotted by the circles.

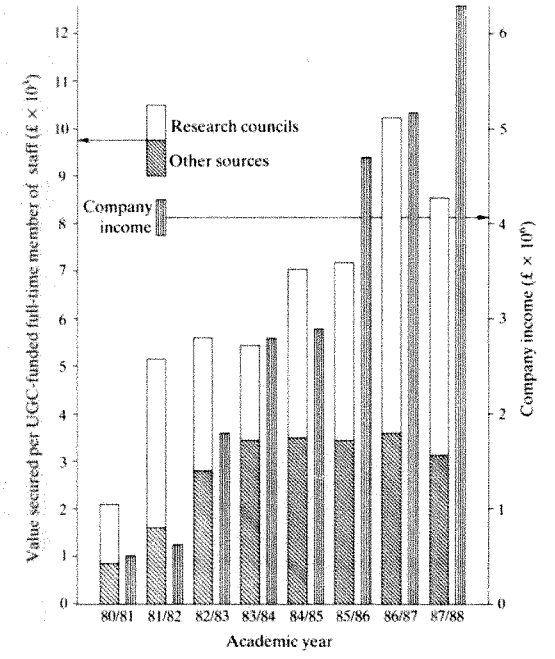


Fig. 2 Research grant and contract income of University of Salford staff, and gross income of the university's wholly owned companies, between 1980 and 1988.

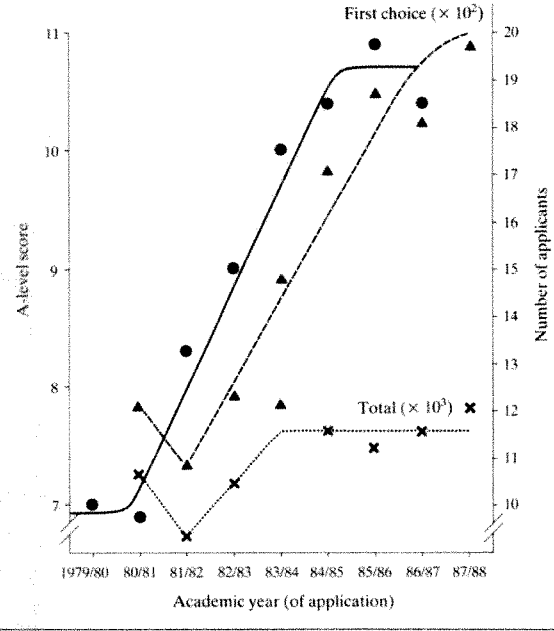


Fig. 3 Characteristics of undergraduate entrants to the University of Salford, and of applications for entry, between 1979 and 1987. Average A-level score of all subjects offered by entrants (circles) is calculated by assigning a value of 5 to an A+ grade down to 1 for an E+ grade, and then dividing the sum by the number of entrants. Total number of applications through the Universities Central Council for Admissions (UCCA) is plotted by the crosses, and number of those who selected Salford as their first choice, of five universities, by the triangles.

emphasis of the university's staff away from a preoccupation with teaching students to a concentration on supporting their capacity to learn — away from an expectation that the university teacher's duty is personally to carry out the teaching, to a realization that the job is much more managerial in nature. Moreover, the success of the university in attracting research contracts (Fig.2) meant that although the number of academic teaching staff (those financed by UGC) declined, the number of full-time postgraduate students and research assistants paid for out of that income increased. The changes are shown in Table 1. These categories of staff and student have been increasingly involved in teaching.

During the 1980s there has thus been a conscious change in the ethos of the university which has profoundly affected the nature of the teaching offered to undergraduates. The best measure of this change is, I believe, the changing ratio of undergraduates to graduates (of all kinds). The ratio changed, as is shown in Table 1, from 4.5 to 2.9 in the period 1980–1988, but that figure understates the true change; the university now employs many more part-time teachers (many of them former full-time employees who have taken early retirement) and has also been much readier than before to employ industrialists of all kinds in teaching. In 1980–1981 the university spent £46,000 on such part-timers; in 1987–1988 £413,600. The link between teaching and research has thus not been so much individual teacher/researchers as the junior associates whom good researchers are able to employ and who have helped both to change the ethos of the institution and to carry out more of the teaching.

Degree scores

Multiple regression analysis of the degree scores in various departments at different times during the past decade can be used to determine the extent to which the change in A-level grades of the intake and the ratio of undergraduates to graduates have contributed to the changes shown in Fig. 1. Such analyses should not be taken to imply causality or even that the relationship is of the same kind in the many different departments. Among other things, we find:

- In the engineering faculty, 46 per cent of the variance can be accounted for, with the main factor being the ratio of undergraduates to graduates.
- In the science faculty, 55 per cent of the variance can be accounted for, with the main factor being the A-level grades of the intake.
- In the social science and arts faculty, only 13 per cent of the variance can be explained and here, as with engineering, the main factor is the undergraduate to graduate ratio.

The so-called linear subjects, recognizably similar to those taken at school, are found predominantly in the science faculty at Salford, so that these results are not unreasonable.

Over the university as a whole, it is clear that the ratio of undergraduates to graduates has had the main effect (the engineering faculty is the largest at Salford). But nearly half of the variance remains unaccounted for.

One factor which must be important, but which is difficult to quantify, is the motivation of the undergraduates themselves. In Fig.3, the total number of applications for admission to the university through the Universities Central Council for Admissions (UCCA) has been plotted together with the number of applicants placing Salford first (out of the five choices permitted in the UCCA system). Although the total number of applications has been approximately constant, the number expressing a preference for Salford has increased throughout the period. This factor must be correlated to some extent with motivation and, statistically, may account for some of the unaccounted variance. But it would be naive to conclude that there is a direct causal relationship between degree class and university preference as expressed on the UCCA application form.

Unfortunately, a change in the UCCA system has meant that students are no longer expected to express any preference between their five choices, so this kind of analysis cannot be extended beyond 1988.

No other readily available management statistic seems closely correlated with the changes shown in Fig.1 although several (such as library use, frequency of borrowing of books and use of computing time, all measured on a *per capita* basis) have increased significantly during this period. A longer run of data, ideally on a departmental or with an individual student basis, will be needed to carry this analysis further and is planned.

The quality of the degrees awarded by the University of Salford has increased markedly during a period of great upheaval, when the number of experienced academic staff was falling both absolutely and in terms of student to staff ratio, and when the demands placed on staff to obtain support from sources other than the UGC for their research (and other) work were intense. This increase in quality is shown both in the performance of those exceptionally good students who obtain first-class degrees and in the 'average' degree awarded. Increases in quality are seen in every faculty and department, and are clearly the result of institutional changes which have affected everyone.

The UGC and other central planning bodies in Britain rarely take into account institutionally based indicators of performance of the kind reported here. Rather, they consider groupings across disciplines, or 'cost centres', from a national perspective. So such central reviews can rarely detect the changes in institutional ethos which, if the arguments in this paper are accepted, are those most relevant to teaching performance.

Correlation

At Salford, the two changes closely correlated both in timing and extent with the improvements of the quality of the degrees awarded are the improvements of academic performance of entering students and the changing ratio of undergraduate students to graduates of all kinds. The former change is of more significance in the science faculty (departments of mathematics and computing, physics, chemistry and biological sciences) and the latter in the faculties of engineering and social science and arts. Yet for individuals, good A-level grades at entry have been no better predictors of class of degree during the 1980s than at previous times, at Salford and elsewhere[2,3].

It is therefore clear that the main factor underlying the improvements shown in Fig.1 is the change in the ratio of undergraduates to graduates, which in turn is a consequence of the increased effectiveness with which the university's staff have obtained research grants and contracts from research councils and from industrial and other sources. This has been the link between 'good teaching' (as measured in Fig. 1) and research at the University of Salford in the 1980s — together, of course, with the adoption of an explicit philosophy of education ('education for capability') which encouraged and sanctioned the use of the young research assistants and graduate students in the teaching programmes of all departments.

It has been suggested that universities which do not offer the full range of research facilities might be established in Britain[1]. This has been interpreted by some to mean that 'teaching-only' universities may evolve. The evidence presented here suggests that such institutions, if established, would be unlikely to produce good graduates as conventionally defined. On the contrary, it would seem that the best way of increasing the teaching effectiveness of a university in Britain is to encourage the staff to do more research and simultaneously to encourage the adoption of a philosophy of education which places more emphasis on student-centred learning and less on staff-centred teaching. □

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Origin of the anomalous ^{40}Ar–^{39}Ar age of Zaire cubic diamonds: excess ^{40}Ar in pristine mantle fluids

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Cubic diamonds from Zaire show excellent correlations between potassium content and $^{40}Ar/^{36}Ar$ ratio, and between $^{40}Ar/^{36}Ar$ and $^{39}Ar/^{36}Ar$, which can be interpreted to yield an 'isochron' age of about 6 Gyr. Now the discovery of a correlation between chlorine content and ^{40}Ar, together with recent mineralogical and geochemical work concerning the origin of cubic diamonds, strongly suggests that the ^{40}Ar is an excess component which has no age significance, and that the ^{40}Ar and its associated potassium are contained in sub-micrometre inclusions of mantle-derived fluid.

ZASHU *et al.*[1] have reported that ten cubic diamonds from Zaire yielded excellent linear correlations in both ^{40}Ar-K and $^{40}Ar/^{36}Ar$ versus $K/^{36}Ar$ diagrams. If the linear arrays are assumed to be isochrons—which is the basic premise in K-Ar dating—the corresponding ages would be about 6 Gyr, which is older than the age of the Earth. The excellent reproducibility of these results precluded experimental deficiencies; Zashu *et al.* were therefore forced to suggest that either: (1) the ^{40}Ar in the diamonds is excess ^{40}Ar and has no age meaning; (2) the basic assumptions underlying the K-Ar dating method, such as the constancy of the $^{40}K/K$ isotope ratio and of the decay constant λ_e of ^{40}K, were not satisfied; or (3) the diamonds or the inclusions in the diamonds are pre-solar. In regard to the second possibility, Akagi and Masuda[2] and Podosek *et al.*[3] analysed Zaire cubic diamonds from the same group used for the K-Ar dating, and found normal K isotope composition. These results also rule out the possibility of a change in λ_e, leaving only the possibilities of excess ^{40}Ar (1) or a pre-solar origin (3). We applied ^{40}Ar-^{39}Ar step-heating dating to four Zaire cubic diamonds from the same group as those used in the previous K-Ar dating[1] and K isotope studies[2,3]. The results showed excellent correlation among ^{40}Ar, K and Cl. Correlation of Cl with ^{40}Ar, together with recent mineralogical and geochemical work concerning the origin of cubic diamonds[4,5], strongly suggests that the anomalous ^{40}Ar and its associated potassium are contained in sub-micrometre inclusions of mantle-derived fluid.

Samples and experimental details

The samples are cubic diamonds from Zaire and are believed to come from the same group as those in the previous studies[1-3]. All the samples show a very regular cubic shape of ~4 mm on a side, but their colours differ from dark brown to brownish, yellow and almost white. Two stones from the same batch were cut in half. Cathodoluminescence images of the cut sections revealed an internal zoning consisting of a coat and a core. This structure is quite similar to those reported on several cubic diamonds by Boyd *et al.* (Fig. 1 in ref. 5).

Diamond samples were irradiated by a total neutron flux of $\sim 3 \times 10^{18}$ neutrons per cm^2 (irradiation parameter $J = 1.709 \times 10^{-3}$ for sample 87N01 and $J = 1.91$–1.83×10^{-3} for samples 88N01-03) with standard samples MMHb-1(6), K_2SO_4 and CaF_2. Before the stepped-heating analysis all samples were heated to 200 °C for 6 hours in an attempt to remove adsorbed atmospheric argon. Except for sample 87N01, for which only two temperature fractions were taken, four temperature fractions were taken for each sample: the samples were kept at each temperature step for 30 min for noble-gas extraction. The experimental procedures for noble-gas extraction, purification and mass-spectrometric analyses are similar to those described previously[7,8].

Results

One of the conventional ways of plotting ^{40}Ar-^{39}Ar data is in the form of 'age spectra'. Because more than 90% of the argon from the diamonds is released in a single temperature step around 1,900 °C, at which point the diamonds are graphitized, there is little to be gained by presenting the data in this form except to note that in most instances the individual ages agree, within error, with the previously reported K-Ar isochron ages of ~6 Gyr. We choose instead to present the more informative isotope-correlation plots[9]. The first of these, Fig. 1, is a conventional isochron plot of $^{40}Ar/^{36}Ar$ against $K/^{36}Ar$. We have adopted the practice of plotting molar ratios, as this seems the most logical way of comparing elemental ratios with isotope ratios (which are invariably expressed in molar terms). The linear correlation in Fig. 1 suggests that ^{40}Ar is derived from sites occupied by K, implying that it is an indicator of age. The age

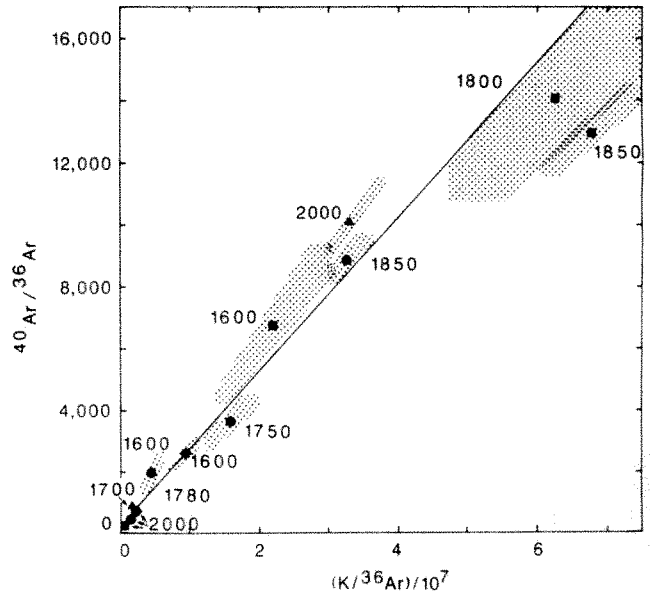


Fig. 1 $^{40}Ar/^{36}Ar$ versus $K/^{36}Ar$ isochron plot. The ^{40}Ar-^{39}Ar age inferred from the slope of the line corresponds to 5.7 Gyr. Symbols represent different samples: ▲, 87N01; ●, 88N01; ■, 88N02; ◆, 88N03. Numerals indicate temperatures (°C). Shaded areas indicate an approximate 1σ error envelope.

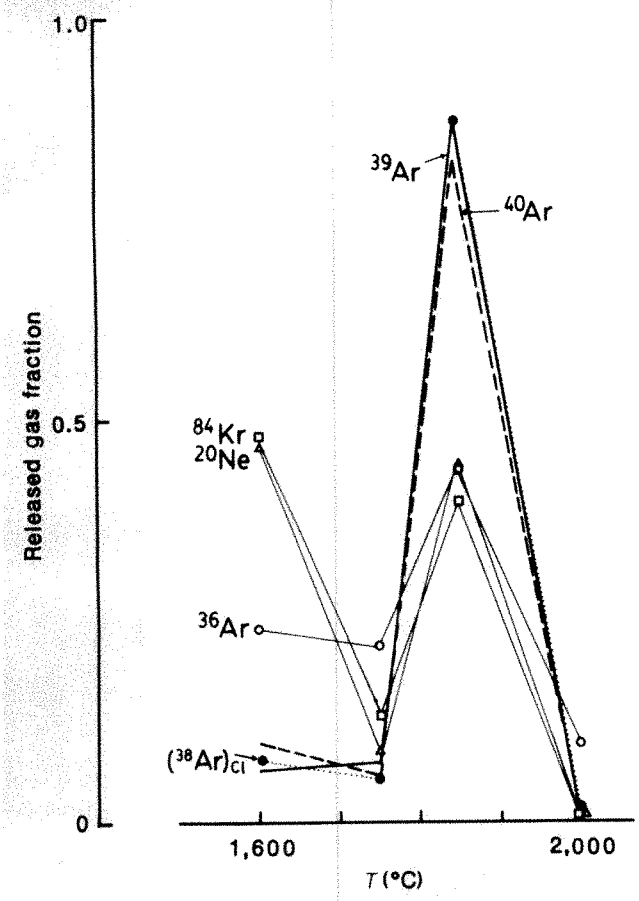


Fig. 2 Thermal-release patterns of noble gases for sample 88N01. $(^{38}Ar)_{Cl}$ indicates ^{38}Ar corrected for trapped ^{38}Ar (equal to 19% of the ^{36}Ar and corresponding essentially to Cl in the diamonds).

calculated from the slope of the line of best fit is ~5.7 Gyr, which is fully consistent with the earlier K–Ar measurements.

In Fig. 2 the fractions of various noble-gas isotopes degassed at each temperature step are plotted against the temperature for sample 88N01. Thermal-release patterns for other samples are almost identical to Fig. 2. The degassing patterns for ^{40}Ar, ^{39}Ar and ^{38}Ar are almost identical, but are distinctly different from those for other noble gases, including ^{36}Ar. This shows conclusively that ^{40}Ar, ^{39}Ar and ^{38}Ar were degassed from similar trapping sites. Because ^{39}Ar is produced by the reaction $^{39}K(n, p)^{39}Ar$, and ^{38}Ar by (n, γ, e^-) reactions of ^{37}Cl, the identical thermal-release patterns for ^{38}Ar, ^{39}Ar and ^{40}Ar indicate that ^{40}Ar was trapped at the same sites as K and Cl. In Table 1 we summarize the analytical data for these ^{40}Ar–^{39}Ar step-heating experiments.

A two-stage growth model

The excellent correlation among ^{40}Ar, K and Cl can be best explained if these elements existed in the fluid phase before having been trapped in diamonds, as elements in such a fluid could be relatively easily homogenized to give rise to a uniform ^{40}Ar:K:Cl ratio. Because of its incompatible nature, ^{40}Ar can be more easily leached from the country rocks into the fluid phase than can be potassium[10]; this would explain the excess ^{40}Ar. If the diamonds trapped such a fluid component to varying degrees, this would give rise to identical thermal-release patterns for ^{40}Ar, K and Cl, and to a linear correlation in the ^{40}Ar–K and ^{40}Ar–Cl diagrams, because they would be trapped as fluid inclusions at the same sites.

Recently, Navon *et al.*[4] studied several cubic diamonds, including two from Zaire, with ion-microprobe and electron-probe analysis, scanning electron microscopy and infrared spectrometry. They observed that coats in the cubic diamonds contain sub-micrometre inclusions which are characteristically enriched in K_2O(8–22%), CaO(6–18%) and other trace elements. The micro-inclusions are composed of 40–70% metal oxides (SiO_2, K_2O, FeO and CaO and smaller amounts of Na_2O, MgO, Al_2O_3, TiO_2, Cr_2O_3, P_2O_5 and Cl), 20–50% water and 10–30% CO_2. Because micro-inclusions were observed commonly in cubic diamonds from various locations, Navon *et al.* suggested that the materials in these micro-inclusions may be related to pristine mantle fluids, which are responsible for widespread metasomatism in the sub-continental mantle.

The difference between the thermal-release patterns of ^{40}Ar, ^{39}Ar, ^{38}Ar and other noble gases (Ne, ^{36}Ar, Kr, Xe) may be explained in terms of a many-stage growth model[5]. Carbon and nitrogen stable-isotope studies suggest that cubic-morphology coated diamonds have undergone two-stage growth: initially a transparent core with octahedral morphology grew in a similar way to common diamonds, and was rimmed by a cubic-habit coat which grew later in contact with the proto-kimerlite host. We suggest that the cores trapped noble gases from the mantle, and that micro-inclusions were incorporated later into the coats during their rapid growth in the presence of fluids. Consequently, the diamonds contain two generations of noble gases: those trapped in the core consist mainly of the normal mantle components, and those contained within micro-inclusions in the coats comprise the excess and *in situ* radiogenic ^{40}Ar.

Table 1 Stepwise thermal degassing of argon isotopes in Zaire cubic diamonds

Sample	T (°C)	^{36}Ar (10^{-9})	^{37}Ar (10^{-12})	^{38}Ar (10^{-9})	^{39}Ar (10^{-12})	^{40}Ar (10^{-6})
87N01	1,700	0.45 ± 0.04	ND*	0.137 ± 0.007	17.3 ± 0.9	0.393 ± 0.009
(0.1991 g)	2,000	0.30 ± 0.04	ND	0.783 ± 0.008	208 ± 1	3.03 ± 0.09
88N01	1,600	0.121 ± 0.042	3.6 ± 1.8	0.081 ± 0.016	12.8 ± 2.1	0.239 ± 0.02
(0.2873 g)	1,750	0.038 ± 0.009	2.7 ± 1.3	0.049 ± 0.017	14.2 ± 1.4	0.137 ± 0.01
	1,850	0.225 ± 0.023	26.6 ± 0.4	0.706 ± 0.007	172.2 ± 1.7	1.99 ± 0.02
	2,000	0.051 ± 0.040	1.6 ± 0.4	0.004 ± 0.004	1.7 ± 0.7	0.024 ± 0.001
88N02	1,600	0.036 ± 0.014	5.2 ± 0.6	0.067 ± 0.009	18.3 ± 1.7	0.241 ± 0.01
(0.2875 g)	1,800	0.143 ± 0.032	70.0 ± 21.0	0.636 ± 0.04	206.7 ± 22.7	2.007 ± 0.05
	1,850	0.228 ± 0.020	2.1 ± 1.3	0.053 ± 0.005	5.7 ± 0.9	0.098 ± 0.005
	2,000	0.027 ± 0.020	~0	0.003 ± 0.003	0.17 ± 0.6	0.008 ± 0.011
88N03	1,600	0.136 ± 0.027	1.2 ± 0.5	0.110 ± 0.01	28.6 ± 3.7	0.351 ± 0.018
(0.3063 g)	1,780	0.098 ± 0.021	0.45 ± 0.28	0.039 ± 0.006	11.2 ± 1.5	0.104 ± 0.008
	1,850	0.192 ± 0.021	10.4 ± 0.7	0.777 ± 0.023	294.6 ± 11.8	2.483 ± 0.050
	2,000	0.075 ± 0.012	~0	0.026 ± 0.003	4.0 ± 2.2	0.056 ± 0.005

Units are cm^3 STP g^{-1}.

* The ^{37}Ar peaks were not detected (ND) because of a long time interval (~2 yr) after irradiation. However, the correction necessary for these has negligible effect on the apparent age because the ^{40}Ar content is much larger.

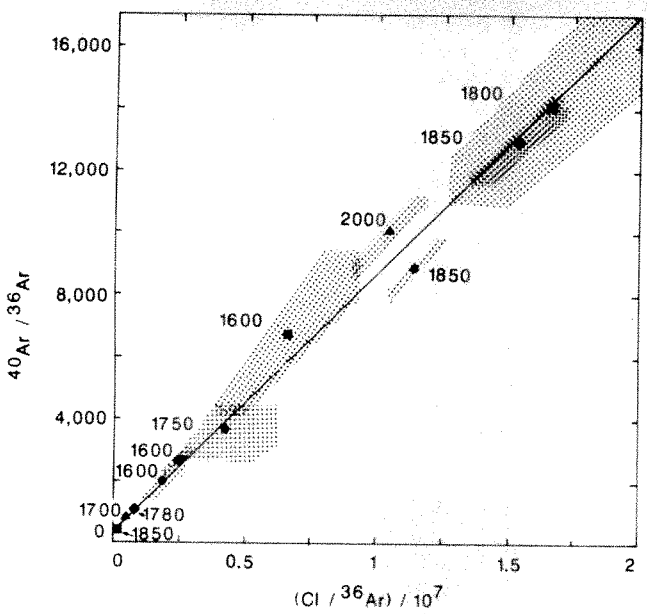


Fig. 3 $^{40}Ar/^{36}Ar$ versus $Cl/^{36}Ar$ correlation plot. Symbols are the same as those in Fig. 1.

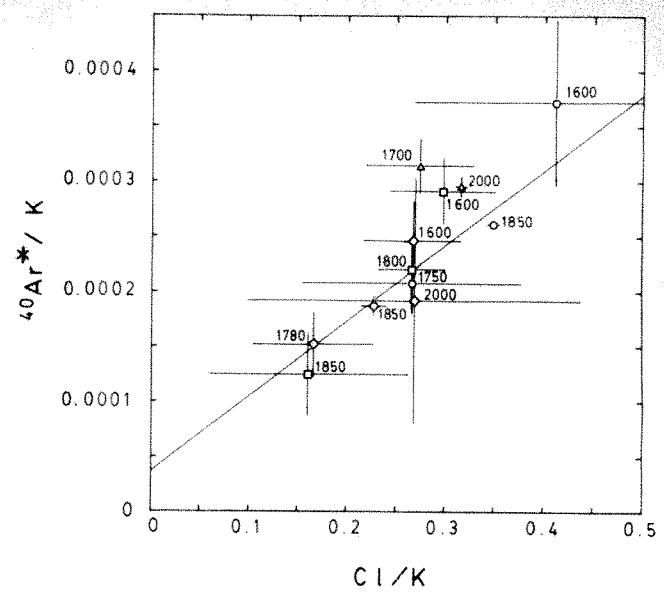


Fig. 4 $^{40}Ar^*/K$ versus Cl/K correlation plot. $^{40}Ar^*$ indicates ^{40}Ar corrected for trapped ^{40}Ar, equal to $340\times{}^{36}Ar$ (the factor 340 is determined from the y-intercept of the line in Fig. 3). Symbols are the same as those in Fig. 1, but are open rather than filled (for clarity).

If the cores crystallized at the deeper region in the mantle and were then transported to the upper mantle where they then developed the coat, the two generations of the noble gas would reflect the respective mantle regions. In fact, such a scenario fits quite nicely the suggestions regarding the noble-gas state in the mantle proposed by many authors[11-14]; whereas the degassed mantle is characterized by a high $^{40}Ar/^{36}Ar$ ratio and thereby a high abundance of ^{40}Ar relative to other noble gases, the less degassed deeper mantle retains the primordial noble gases. The argon that is correlated with potassium and chlorine has a very high $^{40}Ar/^{36}Ar$ ratio, similar to those commonly observed in mid-ocean-ridge basalts and in contrast to the relatively low $^{40}Ar/^{36}Ar$ ratio in the uncorrelated argon (Fig. 2). The latter may correspond to the lower-mantle component. A solar-type neon found in the core of Zaire cubic diamonds[8] thus would also represent the deeper mantle.

The ^{40}Ar–^{39}Ar technique can be used to provide additional chemical information in the form of Ca concentrations, by way of the reaction $^{40}Ca(n,\alpha)^{37}Ar$, and Cl concentrations, by way of the reaction $^{37}Cl(n,\gamma,e^-)^{38}Ar$. Abundances of K, Ca and Cl have been calculated from ^{39}Ar, ^{37}Ar and ^{38}Ar after the usual interference corrections plus a correction for trapped ^{38}Ar (equal to 19% of the measured ^{36}Ar). Bulk element abundances for the four samples analysed, based on a summation of the temperature steps, are given in Table 2. The absolute abundances in the table are probably not particularly significant, in view of the observations by Navon *et al.*[4] that Ca, K and a number of other elements are concentrated, along with H_2O and CO_2, in sub-micrometre inclusions. If the diamonds that we have analysed contain inclusions similar to those observed by Navon *et al.*, we must conclude that the Cl abundances are also extremely high, possibly as much as 1.2–6 wt%.

Table 2 Argon and element abundances

Sample	^{36}Ar (10^{-9} cm^3 STP g^{-1})	^{40}Ar (10^{-9} cm^3 STP g^{-1})	K (p.p.m.)	Cl (p.p.m.)	Ca (p.p.m.)
87N01	0.750	3,423	18.8	5.32	ND
88N01	0.435	2,390	15.0	4.62	5.44
88N02	0.434	2,354	17.4	4.18	12.33
88N03	0.500	2,994	26.0	5.41	1.97

K, Cl and Ca were calculated from ^{39}Ar, ^{38}Ar and ^{37}Ar. The conversion factors for these element abundances are based on measurements of $^{37}Ar/^{39}Ar$, $^{38}Ar/^{39}Ar$ and the J value (from $^{40}Ar/^{39}Ar$) in the monitor MMHb-1, combined with the Ca and Cl abundances determined for MMHb-1 by Roddick[17]. Using the notation of Kelley *et al.*[18], we obtain a production rate of Ar from Ca, relative to the rate from K, of $\alpha = 0.472$, and from Cl, relative to K, of $\beta = 12.2$.

In Fig. 3, $^{40}Ar/^{36}Ar$ is plotted against $Cl/^{36}Ar$. The correlation is excellent and indeed seems superior to the correlation with $K/^{36}Ar$. Figure 3 can be interpreted as a mixing diagram between a component with a low $^{40}Ar/^{36}Ar$ ratio (equal to 340 ± 80 based on the intercept of Fig. 3) and a chlorine-correlated excess ^{40}Ar component with a molar $^{40}Ar/Cl$ ratio of $(8.4\pm0.7)\times10^{-4}$. The situation is analogous to that described in ref. 9 for shallow hydrothermal fluids which have leached Cl and ^{40}Ar from crustal rocks. It is remarkable but not unduly surprising that a similar correlation is found in fluids that we presume to originate in the upper mantle.

Because K/Cl is not constant but shows a factor of two variation (Table 2), $^{40}Ar/^{36}Ar$ cannot correlate perfectly with both $K/^{36}Ar$ and $Cl/^{36}Ar$, and we may therefore investigate whether or not it is possible to separate K-correlated (*in situ* radiogenic?) argon from Cl-correlated (excess?) argon. An attempt to do this is shown in Fig. 4, which is a graph of $^{40}Ar^*/K$ against Cl/K. $^{40}Ar^*$ is the measured ^{40}Ar minus a correction of $340\times{}^{36}Ar$ for the component represented by the intercept of Fig. 3. Because of the high $^{40}Ar/^{36}Ar$ ratios the correction is very small. In spite of the large error bars arising from the small amounts of gas involved, there appears to be a well defined trend in Fig. 4 by which high $^{40}Ar^*/K$ ratios (and correspondingly high apparent K–Ar ages) correlate with high Cl/K. The most straightforward interpretation of the correlation is that it represents a mixture of (predominantly) radiogenic argon in a K-rich phase, $Cl/K\leq0.15$, with (predominantly) excess argon in a Cl-rich phase, $Cl/K\geq0.4$. Under this interpretation, the most meaningful K–Ar age estimate is obtained from $^{40}Ar^*/K$ for the K-rich endmember. It is not possible to determine this ratio with any accuracy from Fig. 4. The best-fit straight line suggests an age between zero and 3.0 Gyr. Although this age is imprecise, it is comfortably less than the age of the Earth.

The data by Navon *et al.*[4] gives a C/K ratio (atomic) of ~9. The Zaire cubic diamonds show fairly constant ratios of $K/^{40}Ar$ (this study and ref. 1) and $^4He/^{40}Ar$ (O.Z. and S.Z., unpublished data), which are 3×10^3 and 0.8 respectively. The $^3He/^4He$ ratios in the Zaire cubic diamonds are in the range $(4.2\text{–}13)\times10^{-6}$ (ref. 8). Hence, we estimate the value of $C/^3He$ in the inclusions

to be $\sim 5 \times 10^9$. This is close to the value of 2×10^9 characteristic of the upper mantle[15,16], thereby suggesting a common occurrence of the fluids in the mantle.

Conclusions

To summarize, we conclude the following. (1) ^{40}Ar-^{39}Ar step-heating dating of four cubic diamonds from Zaire gives an apparent age of 5.7 Gyr, which is close to the K–Ar isochron age obtained earlier[1]. (2) There is excellent correlation of ^{40}Ar with Cl and K in the cubic diamonds (Figs 1 and 3), and thermal-release patterns for ^{40}Ar, K(^{39}Ar) and Cl(^{38}Ar) are almost identical (Fig. 2). These results show that the ^{40}Ar was trapped with K and Cl, most probably as fluid inclusions. Hence, the extraordinary K–Ar age observed for the Zaire cubic diamonds is attributed to the presence of excess ^{40}Ar. The fluid inclusions are likely to correspond to the micro-inclusions observed by Navon *et al.*[4] for several cubic diamonds, including two from Zaire. (3) Most of the other noble gases (Ne, ^{36}Ar, Kr, Xe) reside in different sites to that of ^{40}Ar. These two distinct noble-gases sites can be best explained on the basis of a two-stage growth model for cubic diamonds as proposed by Boyd *et al.*[5]. This model suggests that whereas most of the ^{36}Ar and other noble gases were trapped in the core of cubic diamonds, ^{40}Ar was trapped later as fluid inclusions in the coat of the diamonds in the upper mantle. The solar-like Ne (ref. 8) and the Ar with low $^{40}Ar/^{36}Ar$ ratios, which reside in the core, then represent the mantle region where the core of the diamonds crystallized, which is possibly the deeper mantle.

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Crystal structure of the active site of ribulose-bisphosphate carboxylase

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Ribulose-bisphosphate carboxylase catalyses the key carboxylation reaction of photosynthetic carbon fixation, but also the competing oxygenase reaction of photorespiration. The structure of the active site of ribulose-bisphosphate carboxylase described here should provide a rational basis for attempts to improve the efficiency of the enzyme by genetic engineering.

RUBISCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) is an important target for protein engineering as it controls two competing physiological functions in green plants[1]. The enzyme catalyses the carboxylation step in the Calvin cycle of carbon dioxide fixation, a process that stores the energy trapped by photosynthesis. Rubisco also catalyses the initial oxygenation step in photorespiration, during which a considerable amount of the stored energy is converted to heat thereby limiting crop yield. Detailed knowledge of these catalytic mechanisms as well as the structure of the active site is a prerequisite for rational attempts to modify Rubisco by site-directed mutagenesis to increase the carboxylation/oxygenation ratio.

Both catalytic activities of Rubisco require an activation process during which a lysine residue reacts with an activator CO_2 molecule[2,3]. The labile carbamate formed in this reaction is stabilized by a magnesium ion[4]. The substrate ribulose-1,5-bisphosphate binds to the activated ternary complex and is subsequently either carboxylated by CO_2 or oxygenated by O_2. All biochemical evidence indicates that these two reactions occur at the same site in the protein[1].

We have previously described[5] the folding of the polypeptide backbone of non-activated Rubisco from the photosynthetic bacterium *Rhodospirillum rubrum*. This molecule is a dimer of two identical L-chains, each of relative molecular mass (M_r) 55,000 (55 K), which show about 30% amino-acid sequence identity with the L-chains of Rubisco from higher plants[6]. Almost all side chains are now positioned in this model which has been refined at a resolution of 2.3 Å. Here, we describe the active site of this model as well as the binding mode of phosphoglycerate, which is a product in the catalytic reactions.

Rubisco molecules from higher plants consist of eight L-chains and in addition eight small polypeptide chains, S, (M_r 15K) of unknown function forming an L_8S_8 enzyme molecule. A model, based on X-ray studies, for the arrangement of the subunits in the non-activated form of the enzyme from tobacco has recently been published[7]. We describe here the X-ray structure of the activated form of Rubisco from spinach with a bound transition state analogue, 2-carboxy-D-arabinitol-1,5-bisphosphate (CABP). In addition, we describe the mode of binding of CABP and the active-site metal atom as well as the effector CO_2 molecule and compare the active sites of the non-activated and activated Rubisco molecules.

Structure determination

Rubisco from *Rh. Rubrum*. X-ray data to 1.7 Å resolution of crystals[8] of the non-activated form of *Rh. rubrum* Rubisco were collected on 1° oscillation photographs using the wiggler beam line at the synchrotron radiation source in Daresbury, UK. These

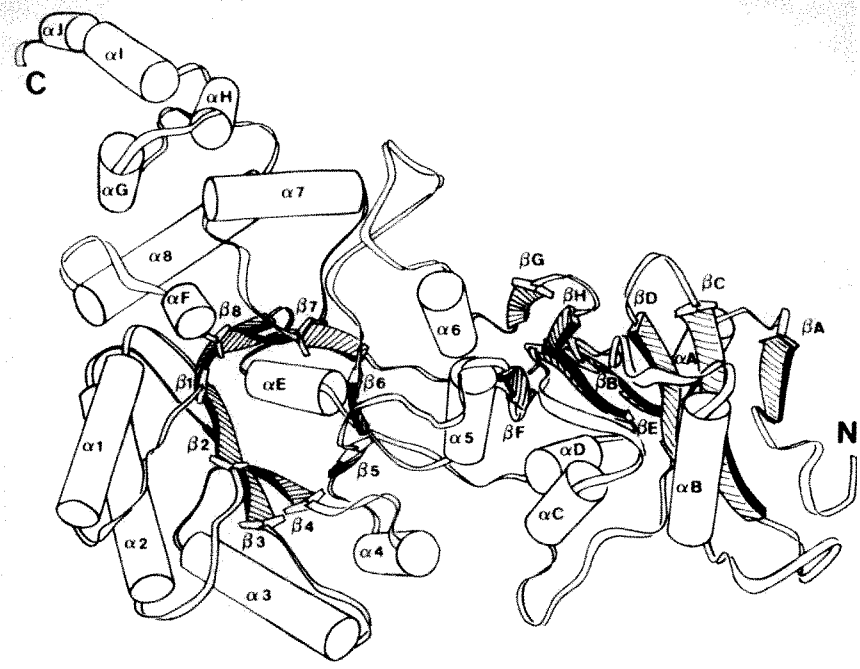


Fig. 1 Schematic view of the large subunit of Rubisco from *Rh. rubrum*. β-strands are represented as striped arrows and α-helices as cylinders. The N-terminal domain comprises residues 1–137 and contains β strands βA–βE, which form a mixed five-stranded β-sheet. Helix A is on one side of the β-sheet, and helices B and C are on the other side of the sheet. A region of extended chain after helix D makes the connection to the C-terminal domain. The C-terminal domain has an α/β barrel structure comprising eight parallel β-strands, β1 to β8, and eight α-helices, α1 to α8 outside the barrel formed by the β-strands. After helix E which closes off the barrel on one side, the chain enters β-strand 1 of the α/β-barrel motif. This domain contains some additional secondary structural features. After helix 6, the polypeptide forms a loop with two antiparallel β-strands (G and H), before the chain enters strand 7 of the α/β barrel. Furthermore, in the loop between β8 and α8 there is a short helix (F), which is not part of the barrel motif. After the barrel motif has been completed the polypeptide chain forms helices G–J at the C terminus. These secondary structure elements are assigned to the corresponding amino-acid sequence numbers for Rubisco from *Rh. rubrum* in Table 2. (Drawing by Ulla Uhlin.)

data were used to refine the initial Rubisco model[5] by the methods of least-squares[9,10] and computer graphics modelling[11]. The crystallographic R-factor decreased from 38.6% at 2.9 Å resolution for the starting model to 21.0% at 2.3 Å resolution for the current model.

Native crystals were soaked with 3-phosphoglycerate and a data set was collected to 2.9 Å resolution on a four-circle diffractometer. The positions of bound 3-phosphoglycerate were determined from a difference Fourier map and the structure of the complex has been refined to an R-value of 19.6%.

Spinach Rubisco. Crystals of activated enzyme which was cocrystallized with the transition state analogue, CABP, were prepared as previously described[12]. Data collection procedures and phasing statistics are presented in Table 1. The L_8S_8 molecule is centered at $x = 0$, $y = 1/4$ and $z = 1/4$ in space group $C222_1$ with a crystallographic twofold axis relating the asymmetric halves of the molecule. The molecule has approximate 422 symmetry as a non-crystallographic fourfold symmetry axis passes through this centre and is inclined at 2.0° to the c-axis in the (101) plane. An envelope of the molecule was defined from an isomorphous, solvent-flattened[13] map, calculated using heavy atom positions located by Pattersson search methods which included the local fourfold symmetry. A final electron density map to 2.8 Å resolution was obtained from phase angles refined by real space averaging[14] of the isomorphous map around the fourfold axis.

A model of the spinach L-chain could easily be built[11] into this map, starting from the *Rh. rubrum* Rubisco structure. The map shows clear density for the activator CO_2 as well as for the transition state analogue, CABP. The metal atom position was located from a difference Fourier map of the cobalt-substituted enzyme.

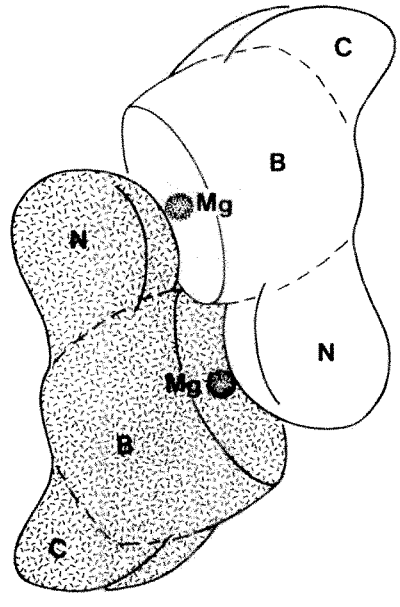


Fig. 2 A schematic diagram of the L_2 Rubisco dimer illustrating that the core of the dimer interface is formed by interactions between the C-terminal domains of both subunits. In addition the N-terminal domain of one subunit interacts with the C-terminal domain of the second subunit in the vicinity of the active sites. (Drawing by Ulla Uhlin.)

Formation of L_8S_8 Rubisco molecules

The subunit structure of the L-chain in L_8S_8 Rubisco from spinach is fairly similar to that of L_2 Rubisco from *Rh. rubrum*. It is divided into two domains, one smaller N-terminal domain linked to a C-terminal domain which has an eight-stranded α/β barrel structure. The domain arrangement in the subunit and the secondary structure of these domains are illustrated in Fig. 1. The amino-acid residues that form the secondary structure elements in the well defined Rubisco subunit from *Rh. rubrum* are given in Table 2.

Two subunits interact tightly to form the functional L_2 dimeric Rubisco molecule of *Rh. rubrum* (Fig. 2). The core of this binding area consists of interactions between the two C-terminal domains around a local twofold axis. In addition, two regions from the N-terminal domain of one subunit interact with regions from the C-terminal domain of the second subunit. These subunit interactions are of functional importance as some of the residues involved occur in or close to the active-site region.

Each active site of the L_2 dimer is thus built up from residues of both subunits. It is therefore not surprising that similar L_2 dimers occur as part of the L_8S_8 Rubisco molecule from spinach as well as tobacco[7] and build up the functional active sites. In the spinach enzyme four such dimers are arranged around the local fourfold axis, building up the L_8 core of the molecule. There are large crevices between the ends of the L_2 dimers at the top and the bottom of the L_8 core. The S-subunits occupy these crevices. The overall shape of the molecule resembles a cylinder with a diameter of approximately 110 Å and height 100 Å. The eight active sites are on the outside of the molecule

Table 1 Data collection, phasing and map averaging

Mean figure of merit: 0.51 for reflections in resolution range 10–2.8 Å

Derivative	(dlim (Å))	Wavelength (Å)	X-ray source*	Number of measurements	Number of reflections	R† (10–2.8 Å)	r.m.s. f_H/E‡
Native	2.4	0.87	SRS	175.242	70.765	0.049	—
$K_2Hg(CN)_4$	2.6	1.49	DESY	125.499	54.917	0.051	1.85
$KAu(CN)_2$	2.4	0.87	SRS	166.141	68.098	0.069	1.41
EMTS§	2.8	1.69	SRS	58.040	32.117	0.082	1.45
Cob§	2.4	0.87	SRS	140.784	65.836	0.044	—

Map averaging statistics at 2.8 Å resolution

	R (%)	r.m.s. (degrees)
Cycle 1	0.41	41
Cycle 5	0.26	58
Cycle 9	0.16	63

Crystals of spinach Rubisco are orthorhombic, space group $C222_1$ with cell dimensions $a = 157.2$ Å, $b = 157.2$ Å and $c = 201.3$ Å. X-ray data were collected on 1° oscillation photographs using synchrotron X-ray sources. Data at 0.87 Å wavelength were obtained from a wiggler beamline. All crystals used were screened for the absence of twinning before use. The data were processed and scaled using the programmes OSC and PROTEIN, kindly provided by J. Remington and W. Steigeman, respectively, and modified by T. A. Jones. Strongly occupied heavy-atom positions were located from difference Pattersson maps using an automated search programme written by one of us (S.K.) which searched both Harker and cross-vectors using the non crystallographic fourfold symmetry of our crystals. The remaining sites were located from difference Fourier maps. For the refinement, 28 positions were used for the Hg derivatives and 12 for the Au derivative. * DESY, The European Molecular Biology Outstation facilities at the Deutsches Electronen Synchrotron, Hamburg; SRS, Synchrotron Radiation Source in Daresbury, UK. (R%), crystallographic R-factor between observed structure factors and calculated structure factors from the averaged map; r.m.s., phase difference between combined phases and mir phases used in the starting map. † $R = \sum |I - \langle I \rangle| / \sum \langle I \rangle$, ‡ f_H, heavy-atom structure factor; E is the residual lack of closure. § EMTS, ethylmercury thio-salicylate; Cob, native crystals grown in the presence of 5 mM $CoCl_2$ instead of $MgCl_2$.

facing the solution. They are spaced widely apart; the shortest distance between the active-site metal atoms is 36 Å. The S-subunits are far from the active sites. There is no contribution to the active site of residues from the S-subunits.

The active site

The active site in the spinach enzyme was identified from the position of the bound transition-state analogue, CABP, as well as the active-site metal atom. The site is shaped like a funnel and is mainly formed by the eight loop regions that connect the eight β-strands with the corresponding helices in the α/β barrel domain. The amino-acid sequences of these loop regions are listed in Table 3 for both *Rh. rubrum* and spinach Rubisco. The N-terminal domain of the second subunit covers part of the top of the active site. In particular, two loop regions of this domain provide residues to the active site. Their sequences are also listed in Table 3.

There is an unusually large number of charged and polar groups in the active site. Within a distance of 10 Å from the active-site metal ion there are four positively charged (Arg and Lys) and four histidine side chains, four negatively charged (Asp and Glu) and 10 other polar side chains which build up one active site region. All the charged side chains as well as the histidines are conserved in all known species of Rubisco. These residues are boxed in Table 3. All of them except one, Glu 48/60, (the first number refers to the *Rh. rubrum* sequence and the second number to the spinach enzyme) are located in the α/β domain.

The position of the active-site metal atom could be accurately determined from the cobalt-substituted spinach enzyme. It is situated in the middle of the active site close to its bottom and is shielded from solution by CABP. Activator CO_2 at the end of the side chain of carbamylated Lys 191/201 forms one of the protein ligands to the metal (Figs 3 and 4). Substrate CO_2 simulated by the carboxyl group of CABP forms a second ligand. In addition, the side chain of Asp 193/203 as well as the hydroxyl group attached to C2 of CABP (Fig. 4) are close enough to the metal to be ligands. A third invariant residue in loop 2 of the

Table 2 Secondary structure elements and the corresponding amino-acid sequence numbers for the *Rh. rubrum* Rubisco subunit

	Secondary structure		Residue number	Secondary structure		Residue number
N-terminal domain	beta	βA	8–10	alpha	α4	268–278
	alpha	αA	14–20	beta	β5	284–287
	beta	βB	23–31	beta	βF	301–303
	alpha	αB	38–49	alpha	α5	305–315
	beta	βC	70–75	beta	β6	318–321
	beta	βD	80–86	alpha	α6	336–344
	alpha	αC	101–109	beta	βG	347–349
	beta	βE	120–127	beta	βH	354–356
	alpha	αD	130–133	beta	β7	364–369
C-terminal domain	alpha	αE	143–150	alpha	α7	376–383
	beta	β1	159–164	beta	β8	389–392
	alpha	α1	173–184	alpha	αF	394–398
	beta	β2	189–191	alpha	α8	403–419
	alpha	α2	204–222	alpha	αG	423–429
	beta	β3	227–231	alpha	αH	431–439
	alpha	α3	237–251	alpha	αI	441–447
	beta	β4	258–263	alpha	αJ	451–455

The labelling of the secondary structure elements is as follows: β-strands and α-helices are labelled in alphabetical order starting from the N terminus except those secondary structure elements which build up the α/β barrel in the C-terminal domain. These eight β-strands and α-helices are numbered β1–β8 and α1–α8. The secondary structure elements were assigned using the algorithm of Kabsch and Sander[28].

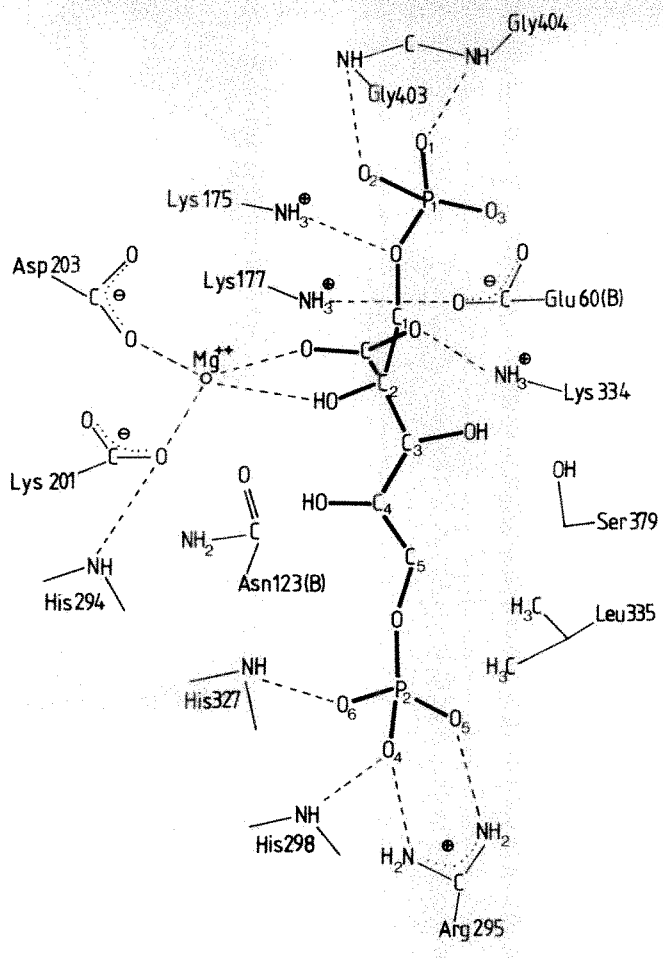


Fig. 3 Schematic diagram of interactions between protein side chains and the metal atom of activated spinach Rubiso as well as a bound transition state analogue, CABP (2-carboxy-D-arabinitol-1,5-bisphosphate).

barrel domain, Glu 194/204, can be model built to ligand the metal through its carboxyl group. The electron density, however, is very weak in our map for this side chain. The invariant residue Asn 111/123 from loop B of the N-terminal domain of the second subunit is also close to the metal atom but too far away (3.5 Å) for strong coordination to the metal atom in our current model.

CABP binds in a rather extended conformation across the barrel (Fig. 4). There are two distinct phosphate-binding sites at opposite sides of the funnel, separating the two phosphorous atoms of CABP by a distance of 9.7 Å. One phosphate-binding site is formed mainly by the adjacent loops 1 and 8 (Table 3). We shall refer to this as phosphate-binding site I. There are two positive charges in the vicinity of the phosphate group, Lys 166/175 and Lys 329/334. In addition two of the phosphate oxygen atoms can form hydrogen bonds to the main chain NH groups of Gly 393/403 and Gly 394/404. These residues are at the N terminus of a short helix in loop 8. Loops 5 and 6 (Table 3) provide the second phosphate binding site II. The side chains of Arg 288/295, His 291/298 and His 321/327 are within 4 Å to oxygen atoms of this phosphate group and surround it with positive charges (Figs 3 and 4).

The sugar bisphosphate molecule is thus anchored at its two ends on opposite sides of the funnel by the phosphate-binding sites and oriented in the middle region by coordination to the active-site metal. In addition the C-4 hydroxyl group interacts with Asn 111/123 from the other subunit (Fig. 4) and the C-3 hydroxyl is close to the invariant residue Ser 368/379. Furthermore, the positively charged groups Lys 166/175 and Lys 329/334 are within interaction distance to the carboxyl group of CABP (Fig. 4) as well as to the phosphate group P1.

We have also studied the binding of the product, phosphoglycerate, to the nonactivated form of the enzyme from *Rh. rubrum*. We find that one molecule of phosphoglycerate binds per active site. When the active site with bound phosphoglycerate is superimposed on the active site of the spinach enzyme with bound CABP we find that the phosphate group of phosphoglycerate binds to phosphate binding site II. Arg 288/295 and His 321/327 provide the positive charges for this binding. The carboxyl group of phosphoglycerate occupies a position close to the position of the metal ion in the activated spinach enzyme. It interacts with the positive side chain of non-carbamylated Lys 191/201. In addition, the asparagine residue from the N-terminal domain Asn 111/123 is also close to the carboxyl group.

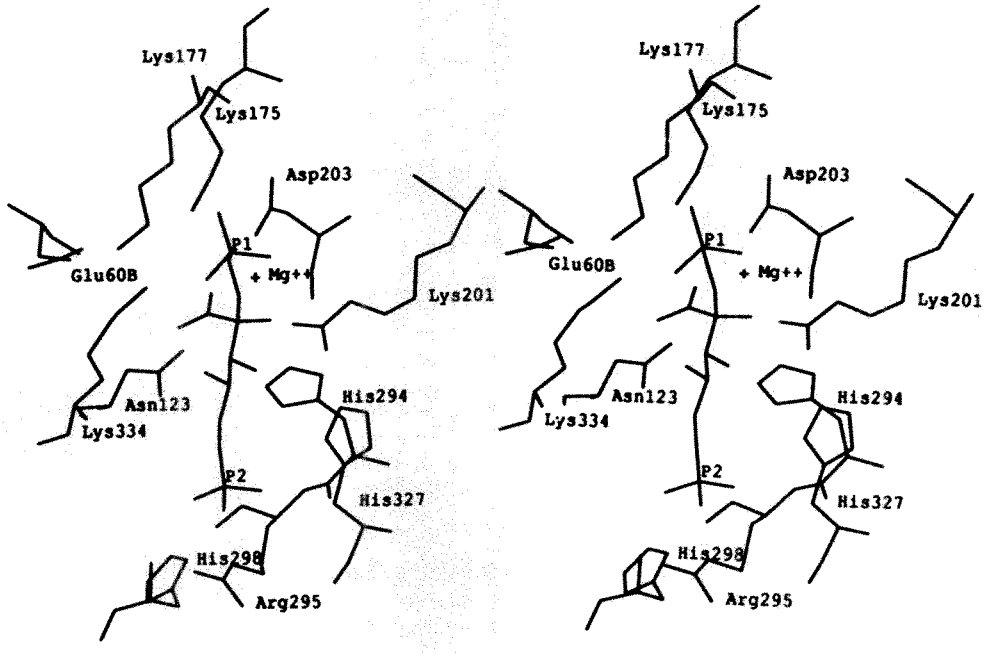


Fig. 4 Computer graphics-generated stereo view of the active site of spinach Rubisco with bound metal atom and CABP.

Table 3 Loop residues which form the active site of Rubisco

C-terminal domain of subunit A

	End of β-strand → 1					Loop								1→ α helix								
					173									182								
Loop 1	—	—	—	C	T	I	K	P	K	L	G	L	S	A	—	—	—					
	—	—	—	T	I	I	K	P	K	L	G	L	R	P	—	—	—					
					164									173								
					201												214					
Loop 2	—	—	—	T	K	D	D	E	N	V	N	S	Q	—	—	—	W	—	—	—		
	—	—	—	I	K	N	D	E	P	Q	G	N	Q	—	—	—	L	—	—	—		
					191												204					
					241						247											
Loop 3	—	—	—	L	N	A	T	A	G	T	C	—	—	—								
	—	—	—	A	N	I	T	A	D	D	P	—	—	—								
					231						237											
					268					273												
Loop 4	—	—	—	H	D	Y	L	T	G	G	—	—	—									
	—	—	—	V	D	G	Y	V	A	G	—	—	—									
					263					268												
					294												310					
Loop 5	—	—	H	I	H	R	A	M	H	—	A	V	—	—	—	—	H	—	—	—		
	—	—	H	Y	H	R	A	G	H	G	A	V	—	—	—	—	T	—	—	—		
					287												304					
					327															347		
Loop 6	—	—	—	I	H	S	G	T	V	V	—	G	K	L	—	—	—	—	—	D	—	—
	—	—	—	I	H	T	G	T	M	G	F	G	K	M	—	—	—	—	—	D	—	—
					321															336		
					380							387										
Loop 7	—	—	—	S	G	G	I	H	V	W	H	M	—	—	—							
	—	—	—	S	G	G	M	N	A	L	R	M	—	—	—							
					369							376										
					402											413						
Loop 8	—	—	—	Q	F	G	G	G	T	L	G	H	P	W	G	N	—	—	—			
	—	—	—	T	A	G	G	G	A	F	G	H	I	D	G	P	—	—	—			
					392											403						

N-terminal domain of subunit B

				57								66										
Loop A	—	—	—	V	A	A	E	S	S	T	G	T	W	—	—	—						
	—	—	—	F	A	A	E	S	S	T	G	T	N	—	—	—						
				45									54									
				118							125											
Loop B	—	—	—	T	S	I	V	G	N	V	F	—	—	—								
	—	—	—	T	L	T	M	G	N	N	Q	—	—	—								
				106							113											

The top two lines of each loop refer to the amino-acid sequence of the spinach enzyme with corresponding residue numbers and the bottom two lines refer to the sequence of the enzyme from *Rh. rubrum*. The numbers of the last residue of the β-strands and the first residue of the α-helices are given. Conserved residues are boxed.

Discussion

The structures described here provide new information on the activation process of Rubisco as well as the identification of a number of specific amino-acid residues in the active site of this enzyme. They also show indirectly that the active-site metal atom is crucial in orienting the substrate molecules for a proper catalytic reaction. Together with biochemical and mutant experiments it can be inferred from this that the metal atom probably also has a more direct role in catalysis by polarizing bonds in reaction intermediates.

Activation is required to form the proper metal-binding site. In the non-activated form Lys 191/201 carries a positive charge and participates in forming a binding site for negatively charged groups as shown in phosphoglycerate binding to the non-activated *Rh. rubrum* enzyme. During the activation process this lysine residue is carbamylated by CO_2 and converted to a negatively charged side chain. The binding site is thereby changed so that it can now accommodate a positively charged metal ion which forms a direct bond to the activator CO_2 group. By binding magnesium, the active site becomes poised to properly bind and orient the substrate molecules for catalysis. At least in the six-carbon reaction intermediate, for which CABP is an analogue, this is achieved by direct binding, both to one of the hydroxyl groups of the sugar bisphosphate and to the substrate CO_2 group.

This latter conclusion is strongly dependent on correct interpretation of the electron density for CABP. The structural model agrees on this point with electron paramagnetic resonance data[15,16] which are consistent with metal binding to both these groups. The orientation of CABP in our model also agrees well with the stereochemistry of the catalytic reaction[17] and with NMR measurements on the relative distances between the metal atom and the phosphorous atoms. In our model the distance from the metal to P2 is 7.8 Å and to P1 6.6 Å. The ratio of these distances, 1.2, is in good agreement with NMR measurements[18]. Furthermore, by making similar NMR measurements on CABP labelled separately on P1 and P2 with ^{17}O it has been shown[19] that P1 is closer to the metal atom than P2.

Several mutants of the metal ligands have been constructed using the *Rh. rubrum* gene to study the functional role of magnesium and its ligands both in the overall reaction and in partial reactions[20]. The results of these studies can now be correlated to the structure of the active site.

To determine whether the charge or the length of the lysine side chain is the important feature of the activation process Lys 191/201 was changed[21] to Glu. The mutant was completely inactive and could not form a stable complex with CABP. Model building shows that Glu is too short to form a proper metal-binding site instead of carbamylated Lys.

Mutation of the metal ligand Asp 193/203 in *Rh. rubrum* to Asn abolishes the carboxylation reaction[20]. At high concentrations of magnesium however, the mutant catalyses formation of product when presented with the six-carbon reaction intermediate (2'carboxy-3-keto-D-arabinitol 1,5-bisphosphate). It did not, however, catalyse an earlier step in the reaction sequence, enolization of the substrate ribulose 1,5-bisphosphate. This early step, which is essential for CO_2 addition and which involves abstraction of a proton from C3 of the substrate thus requires an intact metal-binding site. Enzymatic studies of Rubisco where magnesium has been substituted with different metals have also shown that the nature of the metal strongly influences partitioning between carboxylation and oxygenation[22].

Mutants where the adjacent residue Glu 194/204 has been changed to Gln or Val did not catalyse overall carboxylation, nor the partial enolization reaction or product formation from the reaction intermediate[20]. Clearly this residue has an important function, possibly as a metal ligand.

Identification of the base responsible for the proton abstraction in the enolization reaction has prompted a number of mutant as well as biochemical experiments. Modification of Lys 166/175 by affinity labelling[23] as well as mutations[24] abolishes or drastically decreases catalysis of the enolization reaction. It has therefore been concluded[25,26] that Lys 166/175 is the base responsible for proton abstraction in this partial reaction. In our spinach Rubisco model, which simulates a later stage in the reaction sequence, the nitrogen atom of this side chain is not close to C3 of CABP but instead interacts with the P1 phosphate group and the carboxyl group (Fig. 4). Model building outside the electron density could bring the nitrogen atom within 3 Å from C3. There are, however, four other side chains in our model which are close to C3 of CABP and are also possible candidates for proton abstraction; His 321/327, Lys 329/334, Ser 368/379 and the nitrogen atom of the carbamate group at Lys 191/201.

Glu 48/60 is part of an invariant sequence in loop A of the N-terminal domain. In both structures this residue is involved in subunit interactions with Lys 168/177 from the C-terminal domain of the other subunit (Figs 3 and 4). Mutation of this residue abolishes or drastically reduces activity[27], presumably due to changes in the subunit interactions which could influence the position of Asn 111/123 from loop B of the N-terminal domain which is close to the metal. The active site would no longer be intact and catalysis would be affected.

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LETTERS TO NATURE

Discovery of a double radio source associated with Cygnus X-3

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The combination of high X-ray luminosity[1] (~10^{38} erg s^{-1}), short orbital period[2] (4.8 hours), large non-thermal radio outbursts[3–5] and reports of very-high-energy (up to 10^{15} eV) γ-ray emission[6,7] (see also ref. 8) and muon-rich showers[9–11] makes Cyg X-3 a unique object among X-ray binaries. Very-high-resolution spatial mapping shows that the radio source starts expanding at the beginning of outbursts and becomes elongated[4,5,12], indicating that Cyg X-3 contains a relativistic jet, similar to SS433 (ref. 13). We report here the discovery of a weak (~4.5 mJy), arcsecond-scale double radio source located symmetrically with respect to the compact variable radio core of Cyg X-3. The double radio source has a separation of about 6 arcsec and is roughly aligned with the elongation direction of the sub-arcsec core from Very-Long-Baseline Interferometry observations[12,14,15]. Like the latter it has a rather broad opening angle. This suggests a generic connection between the inner and outer radio structures, and implies that the (poor) collimation evident in the large-scale double source originates near the binary system.

The observations described here were carried out on 14 October 1987 using the Westerbork Synthesis Radio Telescope at 4,874 MHz (6 cm) with an 80-MHz bandwidth, providing an r.m.s. noise level of 0.08 mJy after 12 hours. We did the measurements in standard synthesis mode[16] as part of a long-term study of the variability of the source. In view of this variability (see Fig. 1), we have taken special steps to obtain a map with the required dynamic range. Standard redundancy self-calibration[1] and phase alignment using the unresolved central source enabled us to obtain a dynamic range in the final map (referred to the strength of the central source) in excess of 30 dB. To remove the central component and its near-in sidelobes, we have subtracted a point source from the map at the known position of Cyg X-3 using the flux density given by the measured light curve

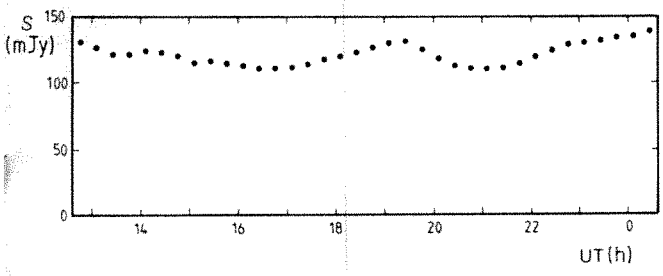


Fig. 1 The 6-cm flux density of the central core of Cygnus X-3 shown as a function of time (UT) on 14 October 1987. Each data point is an average over 20 minutes.

Because our angular resolution is only just sufficient to separate the outer emission from the intense core, there is some uncertainty in the exact level of the central extended emission which underlies the core, and also in the total flux density quoted below. Two other long observations from the present series, of 11 and 9 hours, reproduce the faint double-lobe structure and provide an independent check upon the reliability of our map. The extended structure surrounding Cyg X-3 is shown in Fig. 2. The dominant central component (indicated by a cross) straddles a symmetrical double source with a separation of ~6 arcsec. The axis of this double structure is elongated approximately north-south. The opening angle of the components, when seen from the central source, is about 75°. Despite its low flux density (4.5 mJy in total, split roughly equally between the two components), there is little doubt that the outer double is part of Cyg X-3; its morphology and near-perfect alignment with the central component, and the north-south elongation of the central component on sub-arcsec scales[12,14,15], make the association virtually certain.

For a distance of 12 kpc (ref. 1) we can use the observed angular size, component dimensions and flux densities to derive physical parameters for the Cyg X-3 outer double. To compute the source volume, each component has been approximated by a cone of base diameter 0.4 pc and apex height 0.2 pc. Because of their near-equal flux densities and sizes, we take the components to be alike in all respects and give values only for the entire source. We do not know the spectral index α (defined by the flux density (S)-frequency (ν) relationship $S_\nu \propto \nu^\alpha$) of the double source, but we will assume that the spectrum is non-thermal and hence that the emission is incoherent synchrotron radiation. If we take for α values in the range from −0.5 to −1.1, as observed for the lobes of Sco X-1 (ref. 18) and similar

Table 1 Observed and derived radio-source properties

	Cyg X-3	Sco X-1	SS433
Distance (kpc)	12	0.5	5
S_6, extended emission (mJy)	4.5*	17	100
Size, extended emission (pc)	0.35	0.38	0.12
E_{min}, outer components (erg)	10^{45}	8×10^{40}	10^{43}
B_{eq}, outer components (G)	3×10^{-4}	5×10^{-3}	10^{-3}

* Based on assumed flux densities of 7.6 Jy for 3C147 and of 7.4 Jy for 3C286.

to values found in extragalactic non-thermal radio sources, we find that the minimum energy[19] varies from 0.6×10^{45} to 1.7×10^{45} erg. The parameter values are summarized in the first column of Table 1 (where we assume $\alpha = -0.8$). The extended structures of Cyg X-3 and Sco X-1 are similar not only in their double morphology but also in overall linear size, although the other physical parameters are quite different (Table 1).

Apart from its double structure, the most striking morphological feature of Cyg X-3 is the large lateral extent of the outer emission, which contrasts strongly with Sco X-1 (ref. 20). If the radio lobes are the result of beamed energy transport from the central binary system, then their breadth could be ascribed either to a beam that often changes direction (as is actually observed in the compact radio component of SS433 (ref. 13)) or to one that is intrinsically broad. Observational evidence from Very-Long-Baseline Interferometry (VLBI) investigations is somewhat ambiguous, however. Recent measurements[12] of a low-level flare suggest that the inner sub-arcsec structure, which is directed north-south, has a very large opening angle of ~80°. Although observed on a scale some 5,000 times smaller than the outer components, the VLBI structure subtends essentially the same angle (as indicated schematically in Fig. 2). This would appear to suggest that collimation rather than beam wobble is the cause of the outer components' breadth. VLBI maps made during major outbursts, on the other hand, favour the alternative point of view. Studies of flares observed in October 1983 reveal well-collimated double structures, one[14] elongated in position angle 33° (within but not far from the boundary of the opening angle shown in Fig. 2), and another[15] directed north-south. Thus, although there is evidence for a low degree of collimation during some flare events (to the present time, those of low intensity), beam wobble is observed in well-collimated (and thus far major) outbursts. What is clear is that all of the VLBI structures observed so far are contained within the opening angle defined by the outer components shown in Fig. 2.

In Cyg X-3, as in Sco X-1 and SS433, most of the power is radiated at high (optical and X-ray) frequencies by predominantly thermal emission processes close (10^{11}–10^{12} cm) to the binary system. Although the radio emission is energetically insignificant, only the VLBI components (on a scale of 10^{14}–10^{15} cm) exhibit a morphological link to the outer radio lobes, and we argue that the two structures can be tied together on the basis of a twin-jet model, as has been done for SS433 (ref. 13). We will use the observed and inferred properties of the inner and outer radio emission to investigate the timescale required to generate the outer lobes. The 'quiescent' (slowly varying) emission from the centre of Cyg X-3 has a typical flux density of 200 mJy at frequencies ranging up to 20 GHz (ref. 21). About twice a year[22] there are major (10 Jy or more) outbursts lasting for a few days. The total power output from both

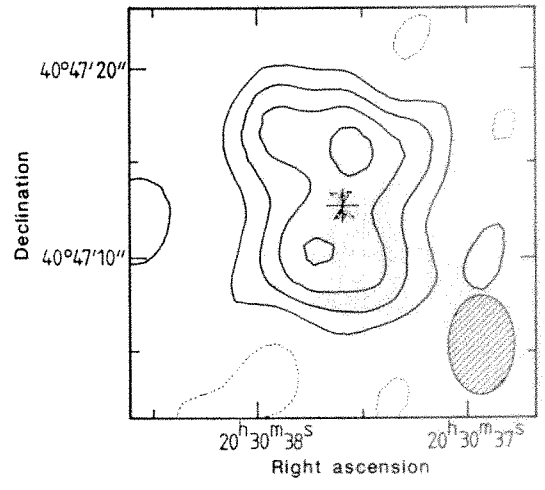


Fig. 2 The extended emission surrounding Cygnus X-3 observed at 6 cm. Contours have been drawn at ±0.21, 0.42, 0.63 and 0.84 mJy per beam. The dominant central component (its position indicated by a cross) has been removed from the map using the flux densities shown in Fig. 1. The hour-glass-shaped shaded region in the centre shows schematically the opening angle of the VLBI components whose true scale is some 5,000 times smaller than the extended structure. The 3.5″ × 5.3″ half-power beam is shown hatched in the lower right-hand corner.

processes (in which the former dominates), P, is $\sim 3 \times 10^{33}$ erg s^{-1}. If a similar amount of power drives the outer lobes then the timescale implied is $E_{min}/P = 11{,}000$ yr (where radiation losses have been ignored, a point we consider below). The corresponding average recession speed of the lobes is nearly 20 km s^{-1}. Although there is no independent evidence as to how energy is shared between the inner and outer radio components, we note that this average speed inferred for Cyg X-3 is similar to that actually measured in one of the lobes of Sco X-1 (ref. 20). The speed at which radio-emitting particles reach the outer lobes may be much higher than this, as implied by the expansion rates of 0.2–0.4c measured using VLBI[14], and by the fact that the components produced by major flares achieve separations of 0.1 arcmin or more after several weeks[14,15].

Radiation losses, which we have ignored in the above estimates, are unlikely to be important provided that the magnetic field strength does not greatly exceed its equipartition value. From standard formulae[19] the lifetime of a synchrotron electron emitting 5-GHz radiation in the equipartition field (Table 1) is 1.4×10^5 yr. This is longer than the age found above, so radiation losses probably do not play a significant role. They do, however, put an upper limit on the age, which could be refined by future determinations of the radio spectrum. Moreover, if the jet efficiency has not changed dramatically with time, Cyg X-3 cannot have been much less active in the past if the outer-component age is to be less than the synchrotron lifetime.

Six of the ten brightest low-mass X-ray binaries (LMXBs) in the Fourth Uhuru Catalogue[23] have been detected as weak radio sources[24]. On the other hand, in a survey of 21 LMXBs by Grindlay and Seaquist[25], only three were detected. These results suggest that radio emission is relatively common in LMXBs but that the radio luminosities vary substantially, a conclusion supported by detailed observations of Sco X-1 (ref. 26), GX13+1 (ref. 27) and GX17+2 (ref. 28). Furthermore, three of the radio-emitting X-ray binaries, Cyg X-3, Sco X-1 and SS433, are now known to have produced extended double radio structures; SS433 also exhibits double-lobed X-ray emission[29]. It is possible that such structures are the rule rather than the exception in LMXB systems, but that they are usually not observed because they are too weak.

The double structure associated with binary systems contrasts strikingly with the morphologies produced and powered by isolated neutron stars. There is no doubt that both the Crab Nebula[30] and the flat spectrum component in CTB80 (refs 31, 32) are maintained by their associated neutron stars/pulsars PSR0531+21 and PSR1951+32, even though their rates of rotational energy loss differ by orders of magnitude. Neither pulsar is in a binary system and both have produced rather amorphous but somewhat elongated synchrotron nebulae. Their structures are quite different from those observed in Sco X-1 and Cyg X-3, a difference which can probably be ascribed to the presence of an accretion disk in the binary systems. Similar arguments are likely to apply to galactic nuclei powering extragalactic double radio sources.

Finally one might wonder why this extended emission was not detected earlier, given that Cyg X-3 has been the subject of intensive scrutiny for over 15 years. One possibility is that, as in Sco X-1 (ref. 20), the outer components vary in intensity. It seems more likely that the extended lobes were not detected previously for instrumental reasons, the major impediment undoubtedly being the strength and variability of the dominant core component. The excellent dynamic range of the Westerbork Telescope was an essential element in our discovery, although the requirements of the present detection (somewhat better than 200:1) are rather modest by the standards of modern synthesis arrays[17]. Our success may derive largely from an angular resolution that happens to be well-matched to the outer lobe dimensions. Unless a significant fraction of the emission in Fig. 2 emanates from sub-arcsecond features, an observation with, say, a 1-arcsec beam would have to reach a noise level of 0.02 mJy and a dynamic range of at least 6,000:1 to obtain a reliable detection. With greater resolution, or when the core is more active or at shorter wavelengths, the demands will be even higher. Variable cores may well be masking similar extended structures in other binary systems.

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Note added in proof: Further analysis of our data suggests that the extended emission is (partially) polarized, establishing its non-thermal nature.

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Is PSR1957+20 eclipsed by a comet, magnetosphere or particulate cloud?

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The extraordinary eclipsing binary pulsar PSR1957+20 is in eclipse over eight per cent of its orbit[1]. This is more than twice the extent of the companion's Roche lobe, the maximum stable synchronous configuration. A possible explanation[2] for the large eclipse is that it is caused by a plasma wind driven from the companion and forming a comet-like tail[3]. But an ionized cometary tail implies a refractive eclipse, which would produce large variations in pulse arrival times; this is inconsistent with the small time delays observed. Here we suggest two alternative explanations. One possibility is that the companion has a typical white-dwarf magnetic field ($\sim 10^5$ gauss), and that the eclipse is then due to the plasma-filled magnetosphere which is confined and blown back sharply by the pulsar wind, with the time delays produced by a much less dense tail. In the other limit of negligible magnetization of the companion, an eclipse could be caused by a particulate cloud (comprising particles of size ~10 cm) which should form

about the companion. We assume that the companion is the remnant of an evolved companion star that is now being excited by the 622-Hz pulsar radiation. Such excitation could either supply plasma to the magnetosphere (for the magnetized case) or pull off neutral gas and deposit it in the vicinity to sustain a particulate cloud (for the non-magnetized case). Near-infrared radiation from this variable system might test the cloud hypothesis, whereas time delays in a magnetized tail suggest radio-frequency polarization tests for the magnetosphere model.

PSR1957+20 consists of a 1.6-ms pulsar and a 0.02-$M_\odot$ companion[1]. The eclipse duration (8×10^{10} cm of orbital trajectory for a pulsar mass of 1.4 $M_\odot$ and therefore a separation $a=1.7\times10^{11}$ cm), is more than a factor of two larger than the Roche-lobe distance (L_1 is located at 2.8×10^{10} cm, and the equipotential surface from that point extends $\pm1.9\times10^{10}$ cm along the orbital axis), so the companion apparently cannot be physically large enough to block the pulsar signal (for this mass, the companion's radius is presumably $\sim3\times10^9$ cm (ref. 4)).

The eclipses of PSR1957+20 by its companion seem quite diagnostic, and seem to be real rather than indicating loss of lock with the pulsar owing to increasing time delay (D. Stinebring, personal communication). For zero orbital eccentricity and an eclipse extending from 0.21 to 0.29 of phase (zero phase is the ascending node and unit phase corresponds to a full revolution), to first order, the eclipse is symmetric and centred on inferior conjunction (that is, as if the pulsar were occulted by the body of an orbiting sphere 4×10^{10} cm in radius). This degree of symmetry rules out almost immediately eclipses of the pulsar by a cometary structure around the companion, because, owing to the intrinsic curvature of such cometary tails, the flanks would be intercepted at different distances and phase angles on entry and exit from eclipse. This is indicated in Fig. 1, where we have numerically simulated the cometary shape; a sharp-entry eclipse would obtain, but not a sharp-exit eclipse. This confirms the sketch given in ref. 3.

In the simulation, we assumed a standard outflow wind of 600 km s^{-1} and radial wind acceleration 12 times the local pulsar gravitational acceleration (chosen only to give a roughly correct cross-sectional size). This model is a direct generalization of a standard one for comets, in which, because of the smaller relative scales involved, the solar-wind acceleration is often approximated as uniform and unidirectional. Of course, this refers to individual particle trajectories, but the overall morphology conforms to the obvious expectations. One can imagine effects that would cause the cometary tail to flare more (plasma pressure gradients within the tail, for example, which must be outward), but not less. E. S. Phinney (personal communication) has suggested that the tail might be highly collimated, expanding only to a fixed cross-section, which would lead to shadowing of the tail by the cometary head; however, shadowing of a relativistic wind would surround the tail with a vacuum region, and rather specialized properties would then be required of the tail plasma for it not to expand to fill these regions, that is, for it not be collimated in the first place. More realistic calculations are clearly needed.

There is a measurable time delay of up to at least 400 μs, which trails off over another 15° following the eclipse, but there is essentially no time delay preceding eclipse. Although it is tempting to suppose that this time delay is directly related to propagation through the tail in Fig. 1, it is too small in magnitude to give a sharp eclipse by having this plasma refract the radio pulses away from the observer. Refraction by plasma occurs away from high plasma concentrations and hence permits eclipses, whereas refraction by neutral gas is towards high gas concentration and hence does not completely interrupt the signal; the Voyager spacecraft was tracked continuously behind Uranus, for example (V. R. Eshleman, personal communication), because of the latter effect. Given that the radius of the system is ~5.8 light-seconds, the average index of refraction along a line of sight that reaches to within 4×10^{10} cm of the companion (the impact parameter at eclipse) differs from unity by less than one part in 10^4, which is much too small to give a refractive eclipse. A refractive eclipse may result (without much time delay) only for a very steep gradient in electron concentration. This could indeed be provided by an advancing cometary structure (corresponding to the entry eclipse in Fig. 1), but would not necessarily give exit from the eclipse at precisely the symmetric phase angle, if eclipse was to come from refraction in the tail; furthermore, one would then expect much larger time delays than are observed.

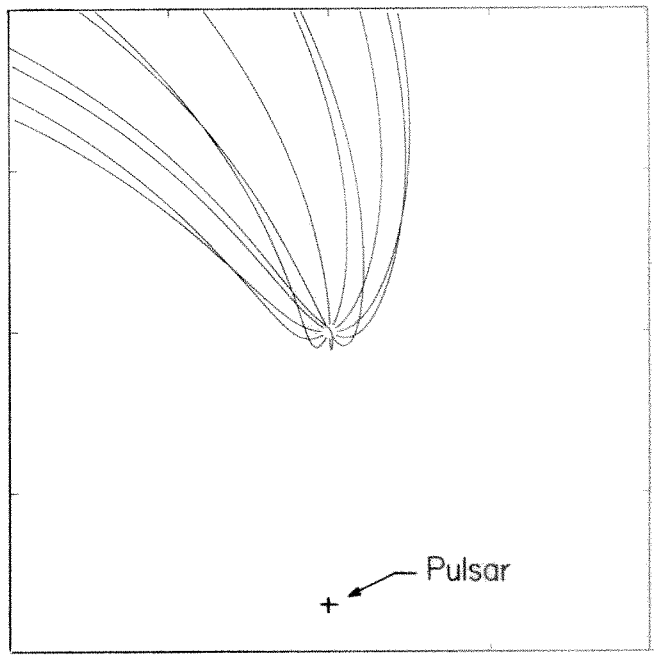


Fig. 1. Simulated cometary wind. Individual particle trajectories are shown in the co-rotating frame, assuming an outflow of 600 km s^{-1} and an outward pulsar-wind acceleration of 12 times the pulsar gravitational attraction (required to give a reasonable scale). Orbital motion is clockwise. Such a system can give a sharp entry eclipse and a sharp refraction exit eclipse, but the exit eclipse would be geometrically unrelated in phase with the entry eclipse, and for refraction to be important the exit eclipse would also have a very large associated time decay. Eclipses are instead observed to be quite symmetric, with only a small time delay.

On the other hand, such eclipses are perfectly consistent with occultation by plasma trapped in an extended magnetosphere. An extended magnetosphere is quite similar in morphology to a comet, with a tight 'nucleus' (the closed field lines) and an extended 'tail' (field lines dragged back by wind interactions). However, the trapped plasma concentration on the closed field lines can be, and usually is, orders of magnitude larger than that on the open field lines, where the plasma is flowing away and being lost. Thus a magnetosphere would present a symmetric occulting object (in contrast to an outflowing cometary flow), and the time delays would be attributable to the small concentrations in the tail (again in contrast to a cometary flow, for which the concentrations in the head cannot be dramatically different from those in the near tail). A significant mass loss does not arise obviously from such a model. The observed maximum column density of 5×10^{16} cm^{-2} is converted to a loss rate by multiplying by some characteristic transverse scale and some characteristic velocity. These are plausibly determined by, respectively, the eclipse size and the escape velocity ($\sim3\times10^7$ cm s^{-1}), which values then give a scale loss rate of 10^{12} g s^{-1}, which is four orders of magnitude smaller than is required for evaporation.

Given that the pulsar-wind field could be 10–30 G at the companion (if $\dot{P}\geq10^{-20}$ s s^{-1}), one needs a comparable field

from the companion at the magnetopause, and hence a surface field of $\sim 10^5$ G, which value is not at all exceptional for white dwarfs. The magnetopause would be about 13 times the size of the companion, which is very nearly the case for the magnetosphere of the Earth and smaller than that of Jupiter.

If the time delay originates in magnetized plasma, there should be a differential time delay that depends on the sign of circular polarization. If the observing frequency (ω) is high compared with cyclotron (ω_c) and plasma (ω_p) frequencies, the time delay $\delta t_\pm$ is

$$\delta t_\pm = \frac{\omega_p^2}{2\omega^2}\frac{L}{c}\left[1 \pm \frac{2\omega_c}{\omega}\right]$$

(where L is the path length), so the difference between left- and right-circular-polarization time-delays is a factor of $4\omega_c/\omega$ of the average time delay ($\sim 400\,\mu$s at 430 MHz). The observed Faraday delay seems to be less than $\sim 2\,\mu$s (D. Stinebring, personal communication), implying that the limiting tail field is ~ 0.2 G.

This value is small compared with that at the magnetopause, whereas one expects that the two should be roughly comparable (within about an order of magnitude). If the magnetic field is inclined relative to the spin axis, however, it is possible that the line of sight is through both lobes of the tail (one with magnetic field away from the companion and the other towards the companion) so that the time delays average out. For the expected radial expansion, these time delays should be established by particles and fields within an orbital radius. Unfortunately the Faraday rotation would be too sensitive a measure of the time delays, giving large rotation measures that would effectively depolarize the radio signal, which is consistent with the observed weak, or possibly non-existent, polarization of the pulsar. Some dependence of frequency on eclipse size would also be expected, being determined by the electron concentration inside the magnetopause.

In the other limit of low magnetization we require a model with characteristics similar to those of Epsilon Aurigae. In ε Aur a cloud of scattering particles is thought to cause occultation, although there are difficult, unresolved questions concerning the particle dynamics, evolution, maintenance and origin. Such questions would also pertain here; however, we have determined numerically that it is at least possible for independent particle ('Kepler') retrograde orbits to exist that extend far enough out from the companion to cause the eclipse. Larger orbits quickly become unstable, suggesting that the size of an occulting cloud may be fixed by orbital stability. One problem common to ε Aur is the requirement for the disk-shaped cloud to be either inclined or thick, as there is a low *a priori* probability of occultation of the pulsar by a thin disk seen edge on. Too thick a disk, however, would obscure optical radiation from the companion. If indeed the companion had no significant magnetic field, the pulsar wind could flow directly onto its photosphere, lighting up the hemisphere facing the pulsar, as observed[5,6]. At the terminator, plasma and entrained gas can be driven away from the companion. The natural consequence would be the formation of a meteor-like trail of ionized gas (which would presumably then produce the time delay) together with a 'fountain' of neutral gas with a wide velocity dispersion. If the companion is indeed the remnant of an evolved star, the vapour-phase fountain will contain some carbon (perhaps predominantly), which is readily condensed and highly refractory. Such a physical system could therefore support an orbiting condensate cloud, with solid particles accreting to significant sizes on each pass of their orbits through the gas fountain. Retrograde orbits are the natural expectation for low-velocity outflow, for which the tail in Fig. 1 is swept back so much that it encircles the companion. Particles several centimetres in size would be required to occult radio signals. About 10^{-10} of the companion's mass, in the form of such particles, would be adequate to cause extinction. If such a disk reprocessed the energy intercepted by the companion, it would constitute a 20th magnitude infrared object at a temperature of $\sim 10^3$ K.

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Evidence from the Semarkona ordinary chondrite for ^{26}Al heating of small planets

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The radioactive decay of ^{26}Al, to form ^{26}Mg, may have constituted an important heat source for melting of small planetary bodies in the early Solar System. Although large excesses of radiogenic ^{26}Mg, arising from *in situ* decay of ^{26}Al (half life $\sim 7.2\times10^5$ yr), have been found in ten carbonaceous and ordinary chondritic meteorites[1,2], the distribution of ^{26}Al in the solar nebula and its importance as a heat source remain to be determined. All previous observations of excess ^{26}Mg are confined to Al-rich minerals in refractory, Ca,Al-rich inclusions (CAIs). An assessment of the distribution of ^{26}Al in the Solar System from these data is difficult because CAIs are only a minor constituent of chondritic meteorites associated with high-temperature nebular processes, and because the initial ^{26}Al/^{27}Al ratio inferred from the CAI data is not constant[3] but varies from the commonly measured value of $\sim 5\times10^{-5}$ to values $<1\times10^{-7}$. Here we report the first observation of radiogenic ^{26}Mg in non-refractory meteoritic material, a plagioclase-bearing, olivine-pyroxene clast chondrule in the Semarkona ordinary chondrite. The inferred initial abundance of ^{26}Al (^{26}Al/^{27}Al $=(7.7\pm2.1)\times10^{-6}$) is sufficient to produce incipient melting in well insulated bodies of chondritic composition. We conclude that planetary accretion and differentiation must have begun on a timescale comparable to the half life of ^{26}Al and that, even if widespread melting did not occur, ^{26}Al heating played a significant role in thermal metamorphism on small planets.

If ^{26}Al was widespread in the nebula and was the heat source for melting small planets, some evidence of this should be preserved in 'old' igneous rocks that cooled on a timescale comparable to the mean life of ^{26}Al. No excesses of ^{26}Mg were found in earlier studies of plagioclase for equilibrated chondrites, achondrites and iron meteorites[4], and from three unequilibrated ordinary chondrites[2]. The preservation of isotope effects is strongly affected by the extent of thermal metamorphism, for which reason the present search for radiogenic ^{26}Mg has focused on the Semarkona chondrite, which is among the least metamorphosed chondrites[5]. Semarkona is a highly unequilibrated, LL-group ordinary chondrite which comprises chondrules and clasts set in a fine-grained matrix containing smectite, calcite and magnetite[6]. *In situ* hydrothermal alteration has selectively affected some chondrules and clasts, suggesting that Semarkona formed in an environment similar to that of the CM2 carbonaceous chondrites and is probably petrological type 2.

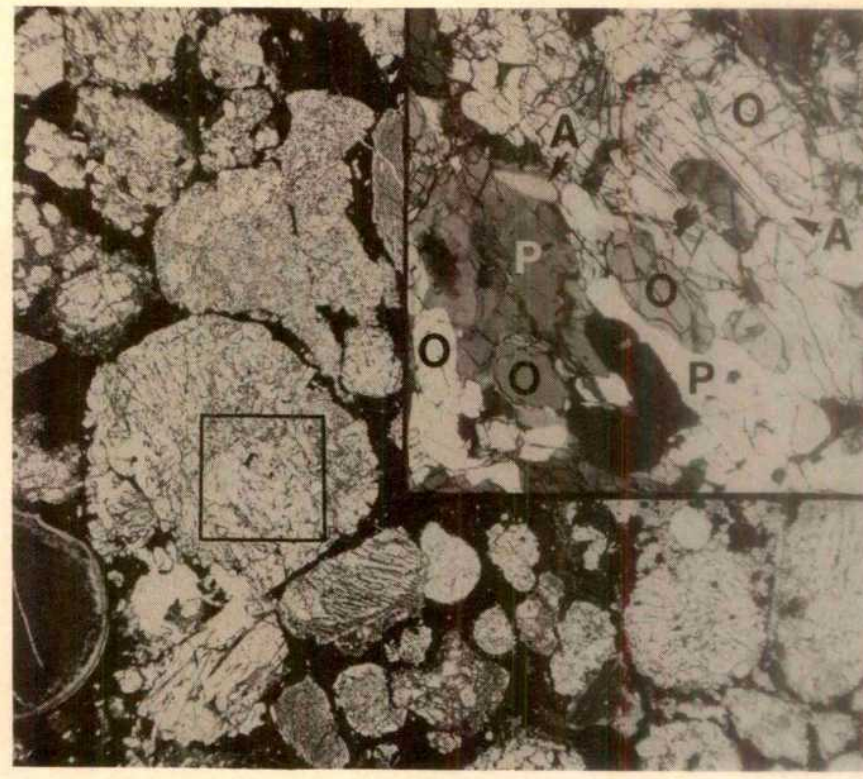


Fig. 1 Optical micrograph (plane-polarized light) of a polished thin section of the Semarkona chondrite. The clast chondrule, CC-1, is indicated by the square; note the roughly pentagonal outline. The square is 500 μm across. Inset shows an enlarged view of CC-1 contained within the square, as seen with crossed polars. Olivine dendrites (O) are poikilitically enclosed in pyroxene (P); anorthite (A) occurs as an interstitial phase.

Anorthite was found in a ~1-mm diameter clast chondrule with a roughly pentagonal outline (Fig. 1). The chondrule, 1805-5 CC-1, is composed of olivine dendrites, some of which traverse the whole chondrule, intergrown with pigeonite. Pigeonite crystals range up to 200 μm in length and poikilitically enclose ovoid portions of olivine dendrites. Crystals of both minerals are truncated at the chondrule margin. The pigeonite may be zoned through subcalcic augite to augite. Anorthite is present as an interstitial phase, typically as 40 × 100 μm laths. CC-1 is free from hydrothermal alteration and contains no glass; ilmenite and kamacite occur as trace constituents. Olivine has a minimum compositional range of Fo_{76} to $Fo_{72.5}$ (here Fo denotes forsterite); CaO and Cr_2O_3 contents are 0.28–0.32 and 0.28–0.55 wt%, respectively. Pyroxene has a minimum range of Wo_8En_{64} to $Wo_{37}En_{48}$, with the corresponding range of 0.49 to 1.43 wt% Cr_2O_3 (here Wo and En denote wollastonite and enstatite respectively). Anorthite contains no detectable Na_2O or K_2O (less than 0.08 and 0.03 wt%, respectively) but contains 0.6–0.8 wt% FeO and 0.5–0.65 wt% MgO. Representative chemical analyses are given in Table 1.

The Mg isotope data were collected with the PANURGE IMS-3F ion probe in an automated peak-jumping mode at a mass-resolving power of 3,300, following techniques discussed by Hutcheon *et al.*[7]. Mass-dependent isotope fractionation (F_{Mg}) is calculated from the deviations in the $^{25}Mg/^{24}Mg$ ratios measured in olivine and pyroxene in CC-1 relative to ratios measured in terrestrial standards. Variations in the $^{26}Mg/^{24}Mg$ ratio are expressed as $\delta^{26}Mg$, relative to $^{26}Mg/^{24}Mg = 0.13955$, and are calculated after normalizing to $^{25}Mg/^{24}Mg = 0.12663$ to correct for instrumental mass fractionation. The $^{27}Al/^{24}Mg$ ratios were calculated from the $^{44}Ca^+/^{24}Mg^+$ ion ratios using a working curve calibrated against electron-probe measurements of terrestrial plagioclase. Abundances of the rare-earth elements (REE) in CC-1 were also measured with PANURGE using an energy-filtering technique[8] at a resolving power of ~500. Concentrations in olivine, pyroxene and anorthite were calculated from REE secondary-ion intensities normalized to the $^{42}Ca^+$ intensity. Pyroxene from the Angra dos Reis achondrite and a synthetic melilite glass[9] were used as standards.

The Mg isotope data (Table 2 and Fig. 2) for CC-1 show small but clearly resolved excesses of ^{26}Mg in five anorthite crystals and isotopically normal Mg in olivine and pigeonite. There is no evidence for isotopically fractionated Mg in any phase, $|F_{Mg}| \leq 3‰$ AMU^{-1}. Excesses of ^{26}Mg (denoted $^{26}Mg^*$) in anorthite range from $\delta^{26}Mg = 2.5 \pm 1.6‰$ to $4.2 \pm 1.8‰$ and are linearly correlated with the $^{27}Al/^{24}Mg$ ratio measured at each point of analysis. The anorthite crystals exhibit only a limited range of Al/Mg ratios; an extensive search for anorthite with Al/Mg > 80 was unsuccessful. These data are characteristic of the *in situ* decay of ^{26}Al in a system with a uniform initial isotope composition, undisturbed by metamorphic processes. In particular, the line defined by the anorthite data is consistent with isotopically normal Mg, as found in olivine and pyroxene, for $^{27}Al/^{24}Mg \ll 1$. The data from the three coexisting phases define an excellent linear correlation on the Al–Mg evolution

Table 1 Chemical analyses of olivine, pyroxene, and plagioclase and the bulk composition of Semarkona CC-1

	1	2	3	4	5	6
SiO_2	37.4	37.4	51.2	48.7	44.9	46.0
TiO_2	—	—	0.40	0.54	—	0.2
Al_2O_3	—	—	2.35	4.35	34.3	4.2
Cr_2O_3	0.51	0.41	0.49	1.43	—	0.5
FeO	21.5	24.8	17.0	8.70	0.85	13.9
MnO	0.45	0.38	0.53	0.34	—	0.4
MgO	39.1	37.2	22.6	16.9	0.81	30.2
CaO	0.30	0.32	3.91	18.0	20.3	3.6
Sum	99.3	100.5	98.5	99.0	101.2	99.0
Fo	76.1	72.5	—	—	—	
Wo	—	—	8.0	37.1		
En	—	—	64.0	48.4		
Fs	—	—	28.0	14.5		

1, 2, extremes of olivine composition; 3, 4, extremes of pyroxene composition; 5, typical plagioclase; 6, bulk composition. Data are wavelength-dispersive electron-microprobe analyses except column 6, which is the average of 30 energy-dispersive analyses. Fs is ferrosilite.

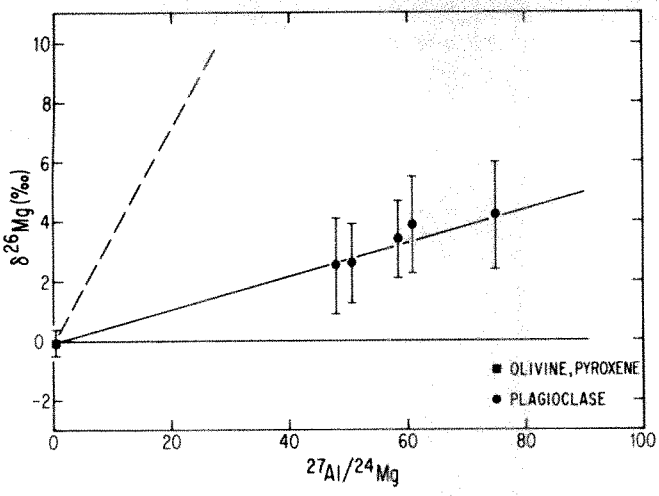


Fig. 2 Al–Mg evolution diagram for coexisting olivine, pyroxene and anorthite in the Semarkona chondrule CC-1. Anorthite contains small excesses of radiogenic ^{26}Mg* clearly resolved from normal Mg. The linear correlation between δ^{26}Mg and Al/Mg is produced by the *in situ* decay of ^{26}Al; the initial ^{26}Al abundance is given by the slope of the solid line through experimental points, $(^{26}Al/^{27}Al)_0 = (7.7 \pm 2.1) \times 10^{-6}$. The dashed line with slope corresponding to $(^{26}Al/^{27}Al)_0 = 5 \times 10^{-5}$ represents the *in situ* decay of ^{26}Al in typical Allende Type B CAI. The horizontal solid line represents normal Mg.

diagram (Fig. 2) with a slope corresponding to $^{26}Mg^*/^{27}Al = (7.7 \pm 2.1) \times 10^{-6}$.

The isotope data from CC-1 demonstrate for the first time that short-lived ^{26}Al was not confined to Ca,Al-rich refractory materials associated with early nebular condensation. This evidence for the *in situ* decay of ^{26}Al in a mafic chondrule from an ordinary chondrite raises two important issues: (1) Does the abundance of ^{26}Mg* in CC-1 provide chronological information on the formation of chondrules? (2) Is the inferred abundance of ^{26}Al sufficient to cause melting of chondritic planetesimals? The initial $^{26}Al/^{27}Al$ ratio indicated by the CC-1 data is approximately a factor of six less than the value of $\sim 5 \times 10^{-5}$ which is characteristic of many Allende type B1 CAIs. If this difference is due to the decay of ^{26}Al from a uniform initial value, then Semarkona CC-1 formed $\sim 2 \times 10^6$ yr after many type B1 CAIs. However, the large variation in $^{26}Mg^*/^{27}Al$ ratios measured in CAIs[3] strongly suggests that the $^{26}Al/^{27}Al$ ratio was heterogeneous in the solar nebula. Differences in inferred $^{26}Al/^{27}Al$ ratios cannot, therefore, be attributed to differences in age. The presence of 'live' ^{26}Al in CC-1 does require that the parent rock solidified very early, within 10^7 years of the time of the ^{26}Al production and not more than a few million years after refractory inclusions.

The interpretation of this evidence of the *in situ* decay of ^{26}Al in terms of the abundance of ^{26}Al in the solar nebula depends critically on the identification of CC-1 as a non-refractory chondrule or fragment produced by igneous activity on a planetary body. The modal mineralogy (olivine 59%, pyroxene 34%, anorthite 7%) and bulk composition (column 6, Table 1) clearly show that CC-1 does not consist of Ca,Al-rich refractory material. The angular form, with crystals terminating abruptly against (rather than nucleating on) the chondrule margin, the mineral chemistry (Table 1) and the exceptionally low Na/Al ratio indicate that CC-1 is a noritic clast chondrule of the poikilitic variety[10], that is, an abraded fragment of an igneous rock and not a quenched liquid droplet. As a group, noritic chondrules have affinities to lunar ANT rocks and to the eucrites[11,12] and most plausibly formed from volatile-poor, differentiated planetary material.

The abundance and distribution of rare-earth elements in CC-1 strongly support a planetary, rather than nebular, origin. The bulk REE content of CC-1 is dominated by the contribution from pyroxene. The REE pattern in high-Ca pyroxene is strongly fractionated, with the heavy REEs (HREEs) enriched; chondrite-normalized abundances (except Eu) increase smoothly from ~0.4 for La to ~5 for Tm. Anorthite exhibits a complementary, HREE-depleted pattern with chondrite-normalized abundances (except Eu) less than unity and a large, positive Eu anomaly ($Eu/Sm \approx 38$). REE abundances in olivine are very low, ≤ 0.01 times chondritic levels for all REEs except La and Yb. The respective REE patterns are characteristic of the partitioning of REEs between basaltic liquid and crystallizing anorthite and pyroxene. The anorthite REE pattern is qualitatively similar to that of plagioclase from the Serra de Magé eucrite[13], although the magnitude of the Eu anomaly in CC-1 anorthite is ~30% greater, comparable to Eu anomalies found in lunar anorthosites[14,15]. The REE abundances in high-Ca pyroxene are in good agreement with predicted REE concentrations based on augite/liquid distribution coefficients[16,17] for a parent liquid with levels of REEs about 8 times those in chondrites.

The mineralogical and chemical evidence argues convincingly for a planetary, rather than nebular, origin for CC-1. The presence of excess ^{26}Mg* in CC-1 indicates that small planetary bodies accreted early and incorporated a substantial amount of ^{26}Al. If CC-1 therefore represents a fragment of early crystallizing material, melting and the production of differentiated liquids must have occurred on a timescale comparable to the mean life of ^{26}Al. During and after crystallization, cooling must have been rapid, and sub-solidus equilibration minimal; otherwise, olivines would have lost CaO and Cr_2O_3 (incorporated into olivine as the primary liquidus phase before pyroxene nucleation) to their neighbouring pyroxenes.

The magnitude of planetary heating caused by ^{26}Al depends primarily on the abundance of ^{26}Al and on the characteristic thermal diffusivity of the body, $\eta = \pi\kappa t/\rho C_p a^2$, where κ is the thermal conductivity, ρ is the density, C_p is the specific heat, a is the planetary radius and t is the time after accretion. For $\eta \ll 1$, the centre of the planet is effectively insulated and most of the heat is retained. In this limiting case, corresponding to $a \geq 30$ km for a compact, chondritic body, the maximum central temperature is linearly related to the ^{26}Al content[4]. For the ratio $^{26}Al/^{27}Al \approx 8 \times 10^{-6}$ inferred for CC-1, ^{26}Al heating will produce central temperatures of ~1,100 °C in a chondritic body. This ratio refers to the ^{26}Al abundance at the time CC-1 became closed to Mg and Al diffusion, and represents a lower limit to the ^{26}Al abundance at the time at which planetary bodies formed. ^{26}Al will be much more abundant and central temperatures will be much higher in bodies that accreted earlier. Incipient melting will occur ($T \geq 1{,}200$ °C) in chondritic bodies accreting only ~70,000 yr earlier, and melting will be widespread in bodies formed more than 100,000 yr earlier.

Detailed solutions to the heat diffusion equation[18] show that,

Table 2 PANURGE ion-probe analyses of the Mg isotope composition of olivine, pyroxene and plagioclase in Semarkona CC-1

Phase	Δ^{25}Mg (‰)†	δ^{26}Mg (‰)‡	$^{27}Al/^{24}Mg$
Plag 1	0.9 ± 1.2	2.5 ± 1.6	48 ± 2
Plag 3	2.5 ± 1.2	2.6 ± 1.4	51 ± 2
Plag 4	1.5 ± 1.3	3.5 ± 1.6	58 ± 2
Plag 5	1.8 ± 1.3	4.2 ± 1.8	75 ± 4
Plag 6	−2.5 ± 1.3	3.9 ± 1.7	61 ± 3
Olivine*	−6.1 ± 0.6	−0.3 ± 1.0	<0.001
Pyroxene*	−1.8 ± 0.6	0.3 ± 1.0	~0.15

* Averages of two analyses of two crystals each. All errors are 2σ.

† Deviation (‰) of unnormalized data relative to $^{25}Mg/^{24}Mg = 0.12663$.

‡ Deviations relative to $^{26}Mg/^{24}Mg = 0.13955$, data normalized to $^{25}Mg/^{24}Mg = 0.12663$.

even if ^{26}Al did not produce widespread melting, it was probably a dominant factor driving thermal metamorphism on small planets. For $^{26}Al/^{27}Al = 8 \times 10^{-6}$, the central 25-km-diameter region of a 40-km-diameter, solid chondritic body ($\kappa \approx 0.007$ cm^2 s^{-1}) will be heated to temperatures exceeding 500 °C. Temperatures will remain at over ~500 °C in the central region for ~1.5–3.6×10^6 yr, depending on the distance from the centre. In a porous, loosely compacted body, the thermal diffusivity is much lower ($\kappa \leq 0.0002$ cm^2 s^{-1}) and temperatures exceeding 500 °C are obtained in the central 8-km region of a 10-km-diameter body. Similarly, the low thermal diffusivity of a thick (~50–100-m) regolith will reduce conductive heat loss and increase interior temperatures relative to the value given above for a solid body.

Estimates of metamorphic temperatures of ordinary chondrites range from ~250–400 °C for type 3 to ~750–950 °C for type 6. Sufficient heat should be generated by decay of ^{26}Al in the interiors of 10–100-km-diameter bodies that accreted early and incorporated ^{26}Al at the level inferred for Semarkona CC-1 to account for the majority of the metamorphic features in types 3, 4 and 5 ordinary chondrites.

Semarkona CC-1 is the first anorthite-bearing chondrule from an ordinary chondrite that we have examined for radiogenic $^{26}Mg^*$. If ^{26}Al was an important heat source, evidence of $^{26}Mg^*$ should be found in similar plagioclase-bearing chondrules in other unequilibrated ordinary chondrites. Hinton and Bischoff[2] failed to detect $^{26}Mg^*$ in plagioclase in Al-rich chondrules from three ordinary chondrites, but excess $^{26}Mg^*$ at the level present in Semarkona CC-1 would not have been detected in their study. A more extensive survey of the Mg isotope composition of plagioclase-bearing chondrules and fragments in unequilibrated ordinary chondrites is required to establish the average initial abundance of ^{26}Al.

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Formation of chemically active chlorine compounds by reactions of atmospheric NaCl particles with gaseous N_2O_5 and $ClONO_2$

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Particulate NaCl and NaBr are known[1,2] to react with NO_2 in air at concentrations of a few parts per 10^6 (p.p.m.) to form the corresponding nitrosyl halides XNO (X = Cl, Br), a process that may contribute to the photochemistry of polluted marine areas and remote regions such as the Arctic[3,4]. Here we report experimental evidence that gaseous chlorine nitrate ($ClONO_2$) and dinitrogen pentoxide (N_2O_5) at p.p.m. concentrations also react with NaCl particles at 298 K, to form Cl_2 and $ClNO_2$ respectively. Thus, the chloride ion in a neutral salt can be transformed into gaseous, photochemically active forms of chlorine at 298 K in a manner analogous to that reported recently for the reactions of HCl in ice with $ClONO_2$ and N_2O_5 at 185–200 K (refs 5–8). This work suggests that reactions of N_2O_5 with NaCl in tropospheric marine areas, and possibly in cold climates where chloride salts are used on roads in the winter, should be considered in assessing the atmospheric chemistry of these regions. Furthermore, if the reactions of $ClONO_2$ and N_2O_5 with NaCl occur also at stratospheric temperatures and if NaCl is present in significant concentrations, these reactions may provide another mechanism for recycling ionic halide ions into gaseous species and hence should be considered in models of stratospheric ozone depletion.

Studies of the composition of particles collected in marine air environments, which are composed primarily of sea salt, frequently have a deficiency of chloride ions, as well as other halides such as Br^-, relative to sodium[9]. This has often been attributed[10,11] to ion-exchange reactions of the atmospheric acids HNO_3 and/or H_2SO_4, forming HCl. More recently, NO_2, at concentrations from a few thousand to a few p.p.m., was shown to react with NaCl and NaBr, forming the corresponding nitrosyl halides and sodium nitrate[1,2,12,13].

Recent investigations[5–8] of heterogeneous reactions relevant to stratospheric ozone depletion, particularly in the Antarctic, have established that the following reactions, which are slow in the gas phase[14–17], occur rapidly when HCl is dissolved in ice:

$$HCl + ClONO_2 \xrightarrow[\text{ice}]{} Cl_2 + HNO_3 \quad (1)$$

$$HCl + N_2O_5 \xrightarrow[\text{ice}]{} ClNO_2 + HNO_3 \quad (2)$$

These reactions serve to recycle the reservoir species $ClONO_2$ and HCl into more active forms of chlorine as a result of the rapid photolysis of the products Cl_2 and $ClNO_2$ in the Antarctic spring.

We show here that, in a manner analogous with reactions (1) and (2), $ClONO_2$ and N_2O_5 react readily with NaCl(s) at 298 K. This suggests that recycling of Cl^- from solids into photochemically active gaseous chlorine compounds is a general phenomenon, rather than being restricted to the HCl/ice system. This may have implications for the chemistry of both the troposphere and the stratosphere.

The reaction of NaCl with $ClONO_2$, prepared using the method of Molina *et al.*[18], was studied using both mass spectrometry (MS) and ultraviolet spectrometry (UVS). The MS apparatus is described in detail elsewhere[1,2,9]. Mixtures of $ClONO_2$ (1.7–19%) in He (ultra-high purity, >99.995%) flowed through a needle valve into a low-pressure system operated at 0.11–0.7 torr. On the low-pressure side, the $ClONO_2$/He passed either through a glass bypass or through a cylindrical cell packed with NaCl. It then flowed into a mass spectrometer (dual-chamber Extrel EMBA II equipped with an 800-Hz tuning-fork chopper, lock-in amplifier and Teknivent Vector/One computer interface). Concentrations of $ClONO_2$ reacting with NaCl were $(3.8–11.9) \times 10^{14}$ cm^{-3}, corresponding to 15–48 p.p.m. if mixed with air at 1 atm. The residence time of the $ClONO_2$ in the NaCl-packed cell is estimated to be ~5–10 s.

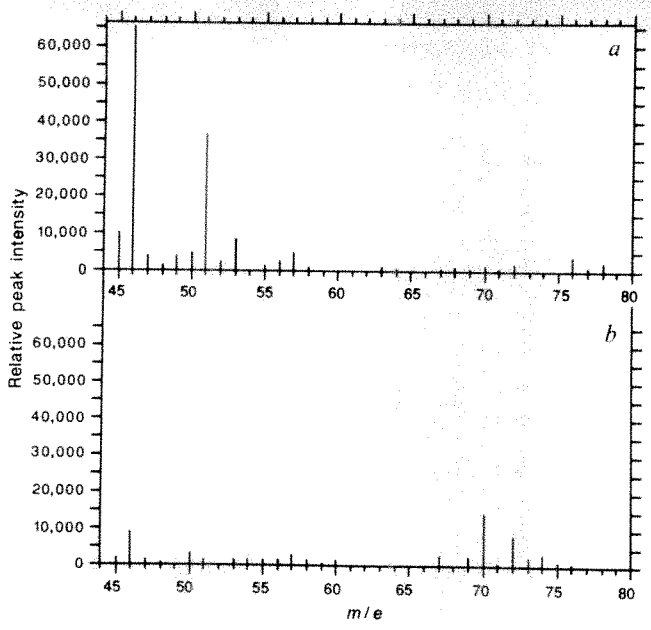


Fig. 1 Mass spectra of $ClONO_2$ when passed through the bypass (*a*), or passed over NaCl at 298 K (*b*). When $ClONO_2$ is exposed to NaCl, the peaks from $ClONO_2$ at $m/e = 46$, 51 and 53 drop significantly and those from Cl_2 at $m/e = 70$, 72 and 74 appear. Initial concentration of $ClONO_2$ was 7.5×10^{14} cm^{-3}; total pressure of He was 0.112 torr. Intensities greater than 65,535 are off scale.

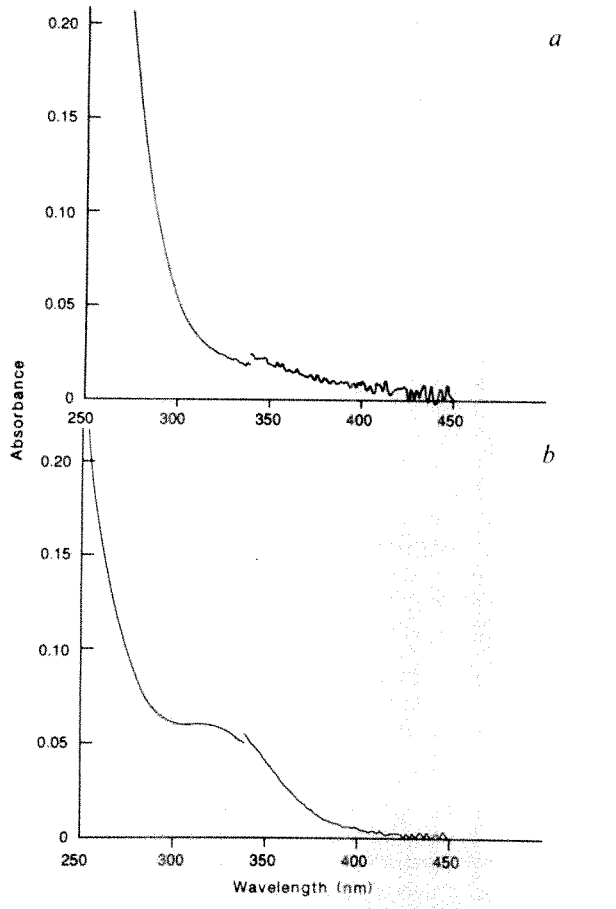


Fig. 2 Ultraviolet spectra of $ClONO_2$: *a*, at 9.2 torr, unreacted, and *b*, at 3.8 torr $ClONO_2$ after reacting with NaCl at 298 K for 71 min. The increased absorbance at ~320 nm is due to the Cl_2 formed in the $ClONO_2$–NaCl reaction.

In these and the N_2O_5 experiments reported below, the NaCl, which was present in excess compared with the gaseous reactants, was heated for several hours under vacuum before exposure to the gases; however, trace amounts of water may be present.

Figure 1 shows the mass spectra in the region $m/e = 44$–80, obtained either when $ClONO_2$ bypasses the NaCl (Fig. 1*a*) or when it flows over the NaCl (Fig. 1*b*). The mass spectrum of the unreacted $ClONO_2$ in Fig. 1*a* shows peaks at $m/e = 46$, 51 and 53 resulting from fragmentation of the parent molecule; it is likely that NO_2 impurity also contributes to the $m/e = 46$ peak. No peaks are seen at $m/e = 70$, 72 or 74, which are characteristic of Cl_2; in independent runs, the Cl_2 impurity in the $ClONO_2$ was estimated to be <0.2%. When $ClONO_2$ passed over the NaCl, however, the peaks characteristic of $ClONO_2$ fell dramatically and those characteristic of Cl_2 appeared (Fig. 1*b*).

Further confirmation of the formation of Cl_2 was obtained using UVS. For these measurements 3.8 torr of $ClONO_2$ in 1 atm air (ultra-high purity, <0.01 p.p.m. total hydrocarbon content, <0.01 p.p.m. CO, <0.001 p.p.m. NO_x, <0.001 p.p.m. SO_2) was allowed to react with ~100 g NaCl in a 5-litre bulb for 71 min. The gas over the salt was then expanded into a 10-cm quartz cell and repressurized with air to 1 atm. Figure 2 compares the UVS spectrum (Fig. 2*b*) of the $ClONO_2$ after reaction with NaCl with that of a higher concentration of unreacted $ClONO_2$ (Fig. 2*a*). The increased absorbance at 320 nm after reaction is characteristic of Cl_2. Using published absorption cross-sections[19], it is estimated that ~40% of the $ClONO_2$ was converted to Cl_2.

Thus both the MS and UVS studies imply the occurrence of the reaction

$$NaCl(s) + ClONO_2(g) \rightarrow Cl_2(g) + NaNO_3(s) \qquad (3)$$

with enthalpy change $\Delta H_0^{298} = -82.8$ kJ mol^{-1}.

In a second set of experiments, N_2O_5 was prepared by reacting NO_2 with excess O_3 and condensing the N_2O_5 at 195 K. Dilute mixtures of N_2O_5 in 1 atm air were expanded simultaneously into the reaction cell and into a 7-litre Fourier-transform infrared (FTIR) multi-pass cell with White cell optics (0.8 m base path, 40 m total pathlength), and both were repressurized to 1 atm with air. The spectrum of the initial N_2O_5/air mixture was recorded using a Mattson Sirius 100 FTIR spectrometer at 2-cm^{-1} resolution with 64 scans. The reaction cell was a 5-litre bulb to which was attached a 200-ml bulb containing ~100 g of NaCl; concentrations of N_2O_5 during the exposure were 2.9–216 p.p.m. The reaction bulb was rotated to thoroughly mix the salt with the gas and then allowed to react in the dark for ~1 hour. The reacted gas mixture was expanded into the FTIR cell, repressurized to 1 atm with air and the spectrum recorded.

Figure 3*a* gives a typical spectrum of the initial reactant mixture in the region 1,300–700 cm^{-1}, showing the bands from N_2O_5 as well as those from some HNO_3 impurity. Figure 3*b* shows the spectrum of the gases after reaction with NaCl for 64 min. The reactant N_2O_5 has disappeared and bands from nitryl chloride[20] ($ClNO_2$) are present, implying the reaction

$$NaCl(s) + N_2O_5(g) \rightarrow ClNO_2(g) + NaNO_3(s) \qquad (4)$$

for which $\Delta H_0^{298} = -55.2$ kJ mol^{-1}. The yield of $ClNO_2$ was approximately proportional to the initial N_2O_5 concentration, as expected if N_2O_5 is the limiting reagent. When small amounts of impurity HNO_3 were present in the reactant, infrared bands from HCl centred around 2,885 cm^{-1} were also observed, as expected for the HNO_3–NaCl reaction[10,11]. Hence, when acid is present, it reacts with NaCl in competition with N_2O_5.

According to reactions (3) and (4), the second product of both reactions should be $NaNO_3$. To confirm this, thin pressed plates were made from the NaCl which had been exposed to either N_2O_5 or $ClONO_2$ and the infrared transmission spectra recorded. Figure 4 shows the spectra of NaCl in the 1,500–800 cm^{-1} region after exposure to N_2O_5 (Fig. 4*a*), after exposure

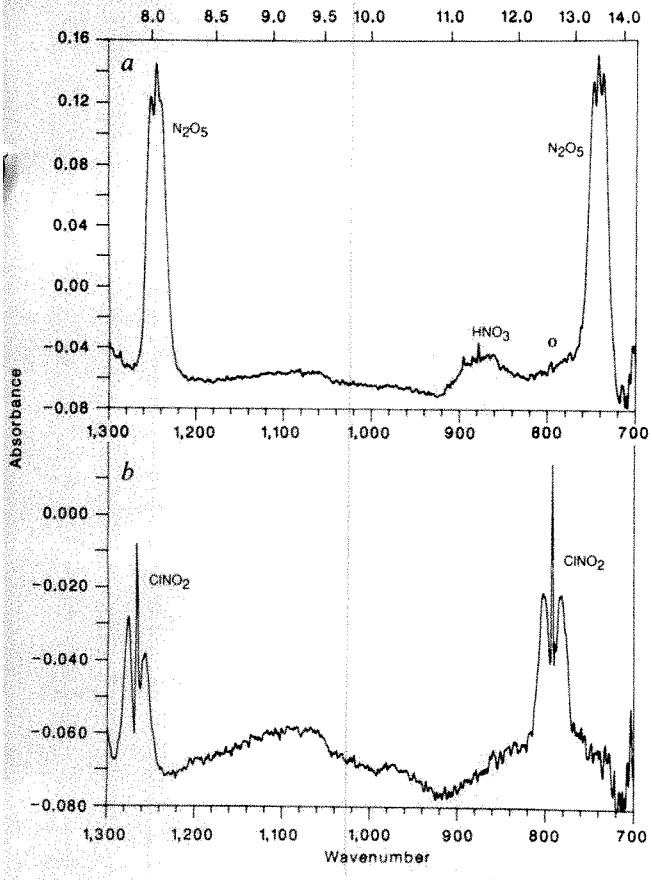


Fig. 3 Infrared spectra of 6.1 p.p.m. N_2O_5 in 1 atm air at 298 K, *a*, before and *b*, after reaction with NaCl for 64 min, showing that $ClNO_2$ is formed.

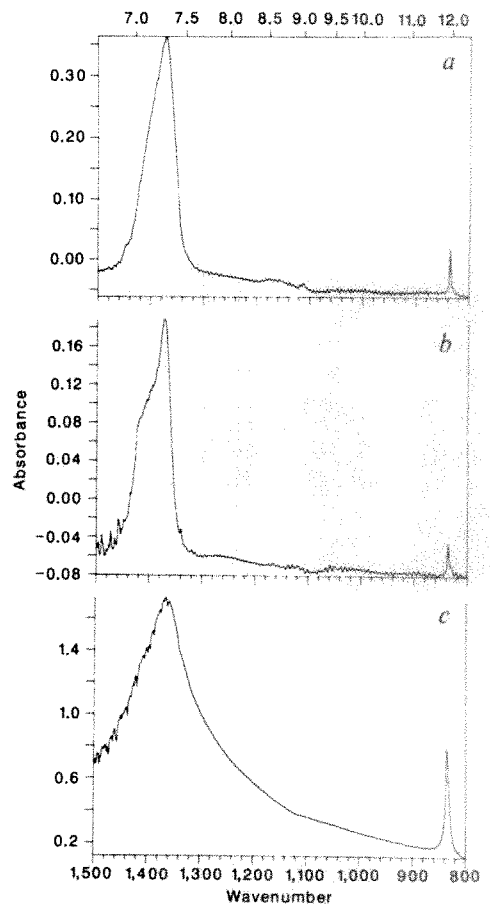


Fig. 4 Infrared spectra of $NaNO_3$ formed in the reactions of NaCl with 1.9 torr N_2O_5 (*a*) and NaCl with $ClONO_2$ (*b*) during mass spectrometry studies (see text). For comparison, *c* shows the spectrum of an authentic sample of a 12.6% $NaNO_3$/NaCl mixture. All spectra referenced to unexposed NaCl.

to $ClONO_2$ (Fig. 4*b*) and for comparison, the spectrum of a $NaNO_3$/NaCl mixture (Fig. 4*c*). Clearly $NaNO_3$ is also a reaction product.

Thus, at room temperature both N_2O_5 and $ClONO_2$ react with solid NaCl in a manner analogous to reactions (1) and (2). In conjunction with our earlier work[1,2], which showed that NO_2 reacts with NaCl and NaBr to form ClNO and BrNO respectively, these studies indicate that recycling of halide ions in salts into gaseous, photochemically active forms can occur through a variety of reactions with species commonly encountered in the atmosphere. The fact that reactions (3) and (4) occur readily suggests that acid catalysis need not be invoked as a necessary condition for the conversion of inactive forms of chlorine into photochemically active gaseous species in either the troposphere or the stratosphere[10,11,21,22].

In the troposphere, the major source of NaCl in particles is as seasalt particles formed by wave action. Thus in polluted marine areas, where NO_2, HNO_3 and N_2O_5 are present, production of ClNO, HCl and $ClNO_2$ may occur, in relative amounts that depend on the concentrations of the gas-phase species and the speeds of the individual reactions. Typical peak concentrations of NO_2, HNO_3 and N_2O_5 in Los Angeles, for example, are ~100 parts per 10^9 (p.p.b.), 50 p.p.b. (ref. 9) and 15 p.p.b. (ref. 23; recent calculations (H. Biermann *et al.*, personal communication) suggest that this N_2O_5 value may be ~8 p.p.b.) respectively. The reaction efficiencies are not known, but we are currently measuring them. However, if the reaction of NaCl with N_2O_5 is approximately an order of magnitude faster than that with NO_2, in heavily polluted marine environments it would compete with the NO_2–NaCl reaction.

Both N_2O_5 and $ClONO_2$ hydrolyse on surfaces on which water is adsorbed[9,24]; hydrolysis of N_2O_5 will thus compete with its reaction with NaCl in the troposphere. Field measurements of NO_3 (which forms N_2O_5 and is in thermal equilibrium with it) indicate, however, that at relative humidities (RH) $\leq$50%, the lifetimes of NO_3 and hence N_2O_5 can be significant in the troposphere[23,25]. The deliquescence point of NaCl is ~75%, so that salt particles formed by wave action will be relatively dry at RH $\leq$ 50% and hence under these conditions the N_2O_5–NaCl reaction may be important.

All studies reported here were carried out at room temperature, but it seems reasonable that these reactions will continue to occur at a significant rate at lower temperatures. Chlorine nitrate is a polar molecule with the chlorine being the positive end; large dipole–dipole interactions with ionic NaCl should lead to the rapid formation of an ion-pair adduct which could rearrange as follows:

$$ClONO_2 + NaCl \rightarrow \begin{matrix} Cl^- & \cdots\cdots & Na^+ \\ \vdots & & \vdots \\ Cl^+ & \cdots\cdots & ONO_2^- \end{matrix} \rightarrow Cl_2 + NaNO_3$$

If there is no activation energy for the reaction, lower temperatures may increase the reaction efficiency by increasing the residence time of the $ClONO_2$ on the surface, as suggested by Molina *et al.*[5] for the $ClONO_2$–HCl/ice reaction.

If both reactions (3) and (4) do occur at significant rates at lower temperatures, they may play a role in the chemistry both of colder regions of the troposphere and of the stratosphere. For example, N_2O_5 could react with chloride salts used on roads

in winter, or indeed with frozen seasalt particles, to form $ClNO_2$, which could then photolyse or undergo other secondary reactions. In addition, by analogy with the studies on NO_2–NaCl and NaBr, other halide salts such as NaBr, if present, would be expected to undergo similar reactions; we are currently studying these systems. Such possibilities should be considered in models of tropospheric chemistry.

Whether NaCl is present in the stratosphere in significant quantities from such sources as meteor ablation or tropospheric injection is subject to some controversy[26–30]. If it is found, our work suggests that reactions (3) and (4) with $ClONO_2$ and N_2O_5 represent other possible mechanisms for recycling Cl^- into active forms. One key factor which may limit the importance of these reactions in recycling any stratospheric NaCl is that once the surface layer has been oxidized to $NaNO_3$, this may effectively prevent further reaction. In contrast, in reactions (1) and (2) the HCl throughout the ice diffuses rapidly to the surface; thus all of the dissolved HCl, not only that on the surface, is available for reaction[5–8].

There are several major caveats in extrapolating our studies to atmospheric, particularly stratospheric, conditions: they were carried out only at room temperature, in the absence of water or other co-pollutants and at higher concentrations than are found in the atmosphere. However, the mechanistic implications for the analogous reactions (1) and (2), which play key roles in stratospheric ozone loss particularly in the Antarctic, are sufficient to argue that the possible roles of reactions (3) and (4) in atmospheric chemistry should be investigated further. We are currently exploring the effects of temperature and water on reactions (3) and (4), and measuring the reaction efficiencies.

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Image processing using light-sensitive chemical waves

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Image processing is usually concerned with the computer manipulation and analysis of pictures[1]. Typical procedures in computer image-processing are concerned with improvement of degraded (low-contrast or noisy) pictures, restoration and reconstruction, segmenting of pictures into parts and pattern recognition of properties of the pre-processed pictures. To solve these problems, digitized pictures are processed by local operations in a sequential manner. Here we describe a special light-sensitive chemical system, a variant of the Belousov–Zhabotinskii medium, in which chemical reaction fronts ('chemical waves') can be modified by light. Projection of a half-tone image on such a medium initiates a very complex response. We are able to demonstrate contrast modification (contrast enhancement or contrast decrease up to contrast reversal from positive to negative and vice versa), discerning of contours (for example, segmenting of pictures up to the extreme case of skeletonizing) and smoothing of partially degraded pictures.

This light-sensitive chemical medium clearly works as a special image processor, and in contrast to sequential computer processes it can be considered to operate as a parallel process. The chemical medium used is a light-sensitive variant of the well-known Belousov–Zhabotinskii (BZ) reaction[2–4]. This reaction is the best studied example of self-organization in nonlinear chemical systems operating far from equilibrium. In homogeneous well stirred solutions, temporal oscillations, bistability and deterministic chaos are observed. In distributed systems, however, if the reaction proceeds in a thin layer of reagent, spontaneous breaking of spatial symmetry occurs. Spatially propagating chemical-reaction fronts (so-called chemical waves) are observed, and two types of waves have been distinguished. If the reagent is in an oscillatory regime and the period of oscillations depends on the spatial coordinate, then a 'phase wave' spreads over the layer. Its velocity is inversely proportional to the phase gradient, as is the case in other physical phase-wave phenomena. The other type of wave, the 'trigger wave', is generated through autocatalytic feedback related to diffusion in the excitable regime. Trigger waves propagate with constant velocity according to a reaction-diffusion mechanism. Chemical trigger waves can be considered as analogous to propagation of flame fronts or of a grass fire. Trigger waves may also occur in the oscillatory regime if a very high phase gradient has been established, as in the case of a chemical system with a high concentration gradient of chemical substances. In such a case the velocity of phase waves is very low and trigger waves can dominate.

Table 1 Compositions of the light-sensitive BZ reagent

	Composition 1 (mol l^{-1})	Composition 2 (mol l^{-1})
$NaBrO_3$	0.3	0.3
H_2SO_4	0.6	0.4
Malonic acid	0.2	0.2
Bromomalonic acid	0.1	0.1
$Ru(bipy)_3Cl_2$	0.0025	0.0025

The BZ reaction occurs between bromate ions and an organic substrate (preferably malonic acid) in acidic conditions, in the presence of a metal-ion redox catalyst[5]. In the light-sensitive variant, the ruthenium bipyridyl complex $Ru(bipy)_3^{2+}$ is used as the catalyst. The main steps in the complicated reaction network of the BZ reaction are autocatalytic oxidation of the catalyst and reduction of the oxidized catalyst by the brominated organic

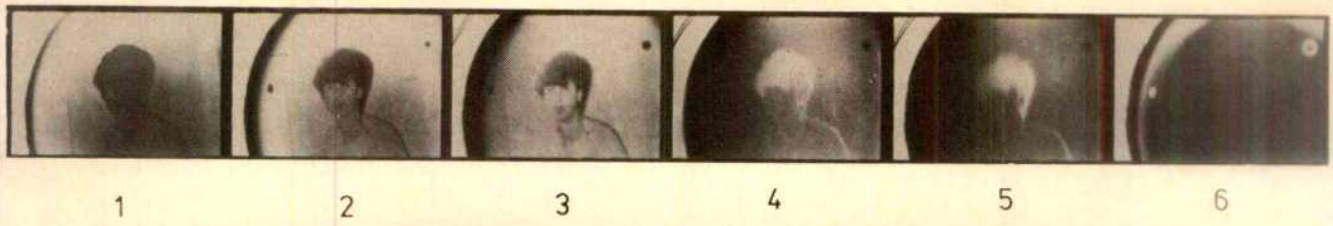


Fig. 1 Light-sensitive BZ reaction (composition 1; Table 1) with a negative image projected onto it. Frame 1: appearance of an image; 2, 3: positive image; 4, 5: negative image; 6: transient phase. The cycle shown here then recurs.

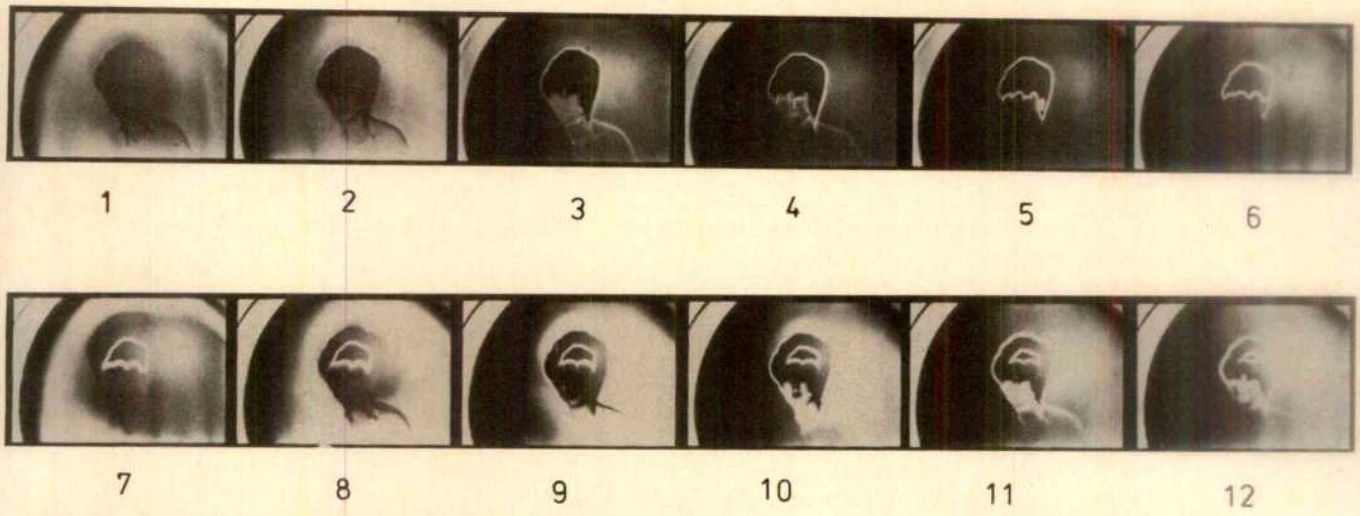


Fig. 2 Image-contour discernment. Same projection as in Fig. 1, but using reagent composition 2 (Table 1). Frames 1, 2: positive image; 3, 4: the dark portion of the image acquires contours; 5–8: erosion of the contour images by trigger waves, which is the mechanism of skeletonizing; 9, 10: formation of a new contour outside the former retracting one; 11, 12: the two retracting contours now fit into one another.

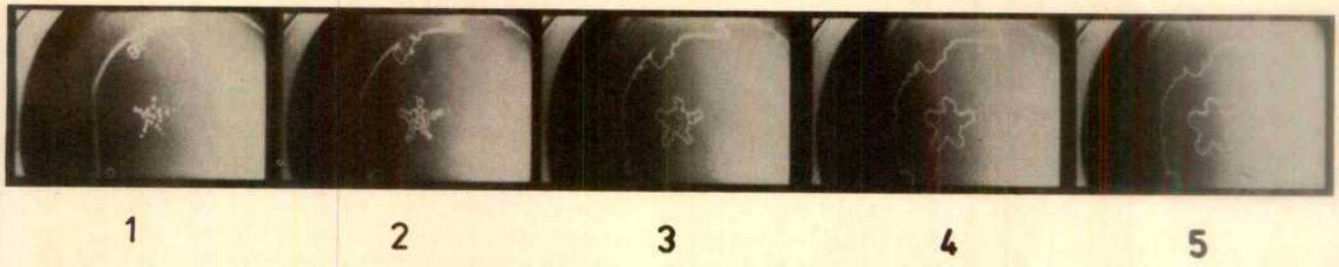


Fig. 3 Smoothing of a discontinuous image. The image, consisting of separate black points against a bright background, was projected on a Petri dish containing the light-sensitive BZ component of composition 2. Frame 1: an image appears as light points against the dark background; 2: each point becomes a source of a propagating concentric wave; 3: waves merge, producing a smoothed image; 4, 5: the image grows in size, and the degree of smoothing increases.

substrate. In the reduction step, liberated bromide ions act as inhibitors to autocatalytic oxidation; thus, bromide is the most important control intermediate and its concentration controls the reaction regime[5,6]. The light-sensitive Ru-catalysed system offers the possibility of selecting the rate of bromide production by photochemical means. By illuminating a distributed system we can control the propagating effects, and, for particular light intensities, observe bifurcations between several reaction regimes. As a consequence, starting in an oscillatory regime and illuminating with increasing light intensity, we can control the velocity of phase waves, transform phase waves into trigger waves, control the velocity of trigger waves and, at very high light intensity, inhibit propagating phenomena completely. By projecting a half-tone image onto a thin layer of reagent, the light intensity is varied as a function of optical density and spatial coordinate. The chemical-wave phenomena are influenced accordingly, and several reaction regimes (for example, phase waves and trigger waves) may be observed in the same layer.

Figures 1–3 show some examples of time sequences of image processing performed by such a system. Fig. 1 demonstrates the possibility of controlling the contrast of an image, Fig. 2 illustrates contour delineation and Fig. 3 shows smoothing of discontinuous images. In these experiments a negative image was projected onto a thin layer (0.8 mm) of the reagent contained in a Petri dish of 9 cm diameter maintained by thermostat at 12 °C. Two reagent compositions were used, distinguished only by acidity (Table 1); composition 1 was used for contrast control and composition 2 for contour discernment and image smoothing. The time interval between the frames in Figs 1–3 was 10 s. In the initially homogeneous solution, there appeared first an inhomogeneity resulting from the influence of the projected image (frame 1 in Figs 1 and 2) and then the positive image itself (frame 2, Figs 1 and 2). For composition 1 (the more acidic solution, in which the oscillatory regime is more pronounced), projection of the negative causes only a change in the oscillatory period, whereas for composition 2 (lower acidity) the same projection causes a transformation from the oscillatory regime

(phase waves) to the excitable regime (trigger waves) in the brightest parts of the image, with the result that contours may be discerned.

In Fig. 1, the change in contrast of the image is accompanied by periodic positive-to-negative transitions, and in Fig. 2 the image contour is revealed. In the latter case, the contour of the brightest part of the image appears first (frame 3), and is then seen to shrink towards the centre of the image (frames 4–8). The process recurs periodically, creating a set of contours inscribed in one another and shrinking successively.

Projecting an image containing discontinuities initiates waves that merge during propagation to cause smoothing of the image (Fig. 3). During the exposure time (t), discontinuities with a length less than $l = \frac{1}{2} vt$ (where v is the propagation velocity of concentration waves in the chemical reaction) will become smoothed.

Let us consider the simplest mechanism of image processing by this light-sensitive reaction. Like the BZ reaction itself, this reaction has an oscillatory character; each point of the system alternates between a fluorescent blueish and an orange colour with a period of ~1–2 min. If a particular arrangement of blue and orange regions is prearranged in the reaction layer (by drawing on the reagent surface with a silver wire), then positive-negative image transitions will be observed (the orange colour being replaced by blue and vice versa) with a period equal to the reaction auto-oscillation period. Such an orange-blue configuration can alternatively be prearranged by projecting onto the medium a half-tone negative (Fig. 4*a*, top). Initially, no image appears in the reaction layer when projection commences, despite the spatial differences in illumination intensity (phase t_1 in Fig. 4). As time progresses, then because the reaction is light-inhibited (and therefore the auto-oscillation period is greater in the illuminated areas than in the non-illuminated areas), the change in colour occurs first in the non-illuminated areas (phase t_2); the initial negative image changes to a positive one. Then, because of auto-oscillations, the reverse takes place (phase t_3), whereupon the process repeats periodically.

Such transitions can be seen in Fig. 1 for a real image with a continuous distribution of illumination intensity. Frame 1 corresponds to the termination of phase t_1, frames 2 and 3 to phase t_2, frames 4 and 5 to phase t_3, and frame 6 again to phase t_1. In this case the contrast changes smoothly from a maximum through zero to the negative region (negative image).

By increasing the range of illumination intensity (or by changing the reagent composition), it is possible to discern the image contours (Fig. 2). In the simplest case, a contour is discerned which is a boundary between auto-oscillating and excitable regions, as illustrated in Fig. 4*b*. The concentration of bromide, c, which inhibits autocatalytic oxidation, determines the reaction mode: for $[Br^-] > c_2$ (Fig. 4*b*), the propagation effects are completely inhibited; for $c_1 < [Br^-] < c_2$, the medium is excitable, and for $[Br^-] < c_1$, the colour oscillates with a period equal to the reaction period (oscillatory regime). In the intermediate regime ($c_1 < [Br^-] < c_2$), propagating waves appear at the boundary of the auto-oscillating and excitable regions (Fig. 4*b*, bottom). A more detailed theoretical description of bifurcations in this reaction system, within the framework of a modified version of the well-known Oregonator model, will be given elsewhere[7].

Note that chemical waves propagating to the centre of an image (Fig. 2, frames 9–12) can be used to discern the image skeleton. Such waves can also be used for image smoothing. Fig. 3 illustrates different stages in the development of a wave pattern resulting from the projection of a fragmentary image. In this case the non-illuminated points serve as leading centres for the creation of new waves, which merge during propagation and result in smoothing of the image. In illuminated areas, trigger-wave propagation is possible but not nucleation of new leading centres; light inhibits the nucleation of new waves.

These results are remarkable for several reasons. First, this system models some operations used in computer image-

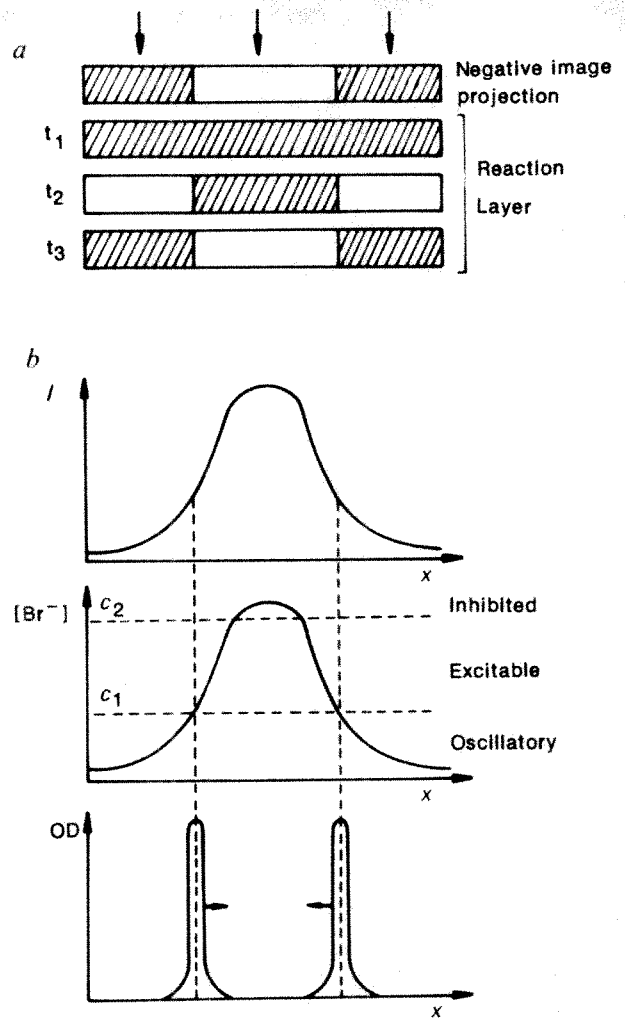


Fig. 4 Mechanism of image processing. *a*, Positive-negative transitions and contrast control. At the top is the negative image projection, and below, three different phases of the reaction (see text). *b*, Contour discernment. Top, illumination intensity of the projected image (I) and corresponding bromide concentration, as functions of the spatial coordinate. Bottom, optical density (OD) in the reaction layer, for the situation in Fig. 2, frame 4 (contouring), which is followed by skeletonizing (frames 5–8).

processing, and might suggest new algorithms (for example, chemical waves can be modelled by cellular automata). Second, there are some speculative connections to visual perception. Contrast enhancement in visual systems is based on the mechanism of lateral inhibition (Mach bands)[8], for example, by neuronal interaction of neighbouring sensor cells. This kind of interaction can also be modelled by molecular diffusion within the framework of a particular chemical model with an autocatalytic step. Third, light-sensitive chemical waves can be seen as a chemical-hardware realization of certain ideas related to new computational systems such as synergetic computers or other network models[9,10] (although in the system described here the interactions are only local). For example, the image smoothing process (Fig. 3) is a realization of an associative memory device: from only an incomplete set of data, the whole pattern may be reconstructed[9]. To construct more sophisticated realizations we require more complex interactions, and in particular long-range interactions. These could be achieved by using special output devices that measure chemical concentration fluctuations at several selected points in the layer. In this way a learning network might be realized by chemical means.

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Depletion of soil aluminium by acid deposition and implications for acid neutralization

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In acid soils acid rain is often largely neutralized by dissolution of aluminium[1-3], which is potentially phyto-toxic[4]. Here we show that only a minor fraction of soil aluminium is readily dissolved. This most soluble fraction consists largely of non-silicate, organically bound aluminium, which has been formed in the course of soil development. The current rapid and irreversible depletion of this fraction constitutes a drastic change in soil genesis. Depletion may eventually result in reduced acid neutralization, as a consequence of decreased dissolution of aluminium, and should be accounted for in soil acidification models.

Dissolution of aluminium in acid soils is one of the most pronounced soil chemical effects of acidic deposition[1-3]. Aqueous aluminium is toxic to crop roots[4-6] and has also been associated with forest die-back[7]. Because of its toxicity the soil chemical behaviour of aluminium has been a major topic in agronomic[8,9] and ecological[10] studies. Most of these studies deal with short-term phenomena such as speciation, exchange characteristics and solubility control. Long-term effects on aqueous aluminium in soils, resulting, for example, from changes in the soil solid phase, are generally ignored. In many acid, sandy soils, however, an appreciable portion of the pools of various solid-phase aluminium fractions is currently leached from the soil. Table 1 indicates that leaching could lead to a significant depletion of certain soil aluminium fractions within several decades. The current depletion rates of extractable aluminium are significantly higher than the rates of aluminium transfer from silicate-bound to extractable soil aluminium. Maximum annual rates of formation of extractable aluminium amount to 0.030 mmol per kg of soil, as estimated from accumulation rates in the soil solid phase[11] (J.M. *et al.*, preprint) and from current rates of organic-aluminium precipitation[11]. Decreasing content of various fractions of solid-phase aluminium could affect the aqueous aluminium concentrations. Insight into these aspects is essential for evaluating long-term effects of acid deposition.

Data on the sources of dissolved aluminium were obtained from a laboratory leaching experiment. Air-dried, <2 mm fractions of soil samples from the Netherlands and New Hampshire, USA, were taken from the surface and sub-surface horizons of two podzols (Haplorthods) and of a recent driftsand (Udipsamment) (Table 1). Duplicate samples of each soil horizon (5.0 g) were leached 10, 25 and 50 times, respectively, with 100 ml aqueous hydrochloric acid at *p*H 3.0. After each acid addition the suspensions were kept in tightly capped centrifuge tubes at 20 ± 2 °C for 24 hours. Suspensions were shaken manually three times in each 24 hours. The suspensions were centrifuged at 2,000 r.p.m. for 15 min, and 80 ml of the clear supernatants was decanted and replaced by 80 ml of fresh HCl solution. Sub-samples of each supernatant were pooled to give a single extract after 10, 25 and 50 HCl treatments. In each case, pooled leachates were analysed for all major solutes, dissolved aluminium concentrations were measured after 6, 24 and 72 hours in one additional leaching, and soil samples were freeze-dried and residual solid aluminium fractions were estimated by sequential selective dissolution (Table 1). Dissolved aluminium was assayed colorimetrically using pyrocatechol-violet.

We selected a leaching solution of *p*H 3.0, because this is close to the current *p*H of the water infiltrating many surface soils of forests and heathlands in north-western Europe[3] (J.M. *et al.*, submitted). We enhanced aluminium mobilization rates relative to those in the field by applying HCl at a rate of 20 mmol H^+ per kg of soil per 24 hours. This is considerably higher than the current acid load in the field (4 mmol H^+ kg^{-1} yr^{-1}).

Table 1 Pools of solid-phase aluminium in three acid, sandy soils from the Netherlands, and one from New Hampshire (USA), before laboratory leaching treatments, and annual aluminium mobilization rates from the respective soil layers in the field

Site	Soil	Depth horizon (cm)	Extractable aluminium* KCl	$Na_4P_2O_7$	$(NH_4)_2C_2O_4$ (mmol kg^{-1})	Total aluminium†	Annual removal of aluminium‡
Gerritsfles	driftsand	0-10 C	2.2	33.3	3.7	450	0.5
		10-40 C	0.7	35.7	6.3	427	0.3
Tongbersven	podzol	0-12 E	1.3	5.0	1.5	101	0.1
		12-35 B	13.7	196	3.3	446	0.4
Hasselsven§	podzol	0- 7 E	11.1	21.2	7.7	221	0.5
		7-22 B	16.3	55.8	13.5	273	0.1
Hubbard Brook	podzol	0- 5 E	1.2	7.8	3.0	1660	0.005
		15-30 B	5.8	322	51.1	2185	−0.004

* Solid-phase aluminium was extracted using selective dissolution. Soil samples (1 g) were sequentially extracted with 50 ml 1M KCl (0.5 hour), 50 ml 0.1M $Na_4P_2O_7$ (16 hours) and 50 ml 0.2M $(NH_4)_2C_2O_4$ adjusted to *p*H 3.0 (4 hours in the dark)[11]. Extracted amounts represent exchangeable aluminium (KCl), organic aluminium and minor amounts of non-crystalline hydrous oxides ($Na_4P_2O_7$) and most amorphous aluminium hydroxides ($(NH_4)_2C_2O_4$)[16,17]. Extracted aluminium was measured using colorimetry with pyrocatechol violet (KCl), inductive coupled plasma spectroscopy ($Na_4P_2O_7$), and atomic absorption spectroscopy with a nitrous oxide-acetylene flame ($(NH_4)_2C_2O_4$).

† Total aluminium, largely originating from aluminium silicates, was determined by X-ray fluorescence spectroscopy of a lithium tetraborate melt of the soil material.

‡ Calculated from bulk density (kg m^{-3}) (H. F. van Dobben and J. Mulder, manuscript in preparation), and estimated aluminium fluxes (kmol ha^{-1} yr^{-1}) (J.M. *et al.*, preprint). Monthly aluminium fluxes were estimated by multiplying the simulated soil water flux and the measured monthly dissolved-aluminium concentration in the soil solutions collected from suction plates in the field. Values for inorganic aluminium in the Hubbard Brook soils were taken from Driscoll *et al.*[11]. Negative numbers indicate immobilization.

§ Not included in the leaching study.

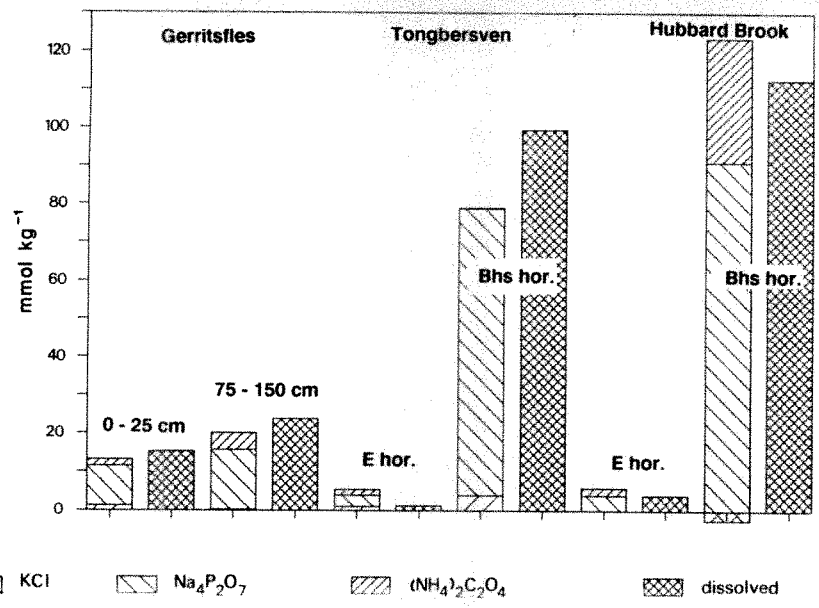


Fig. 1 The decrease in solid-phase aluminium, after 25 leachings with HCl (left bar, hatched) and the amount of aluminium removed in the leachates (right bar, cross-hatched) for six soil samples. Values are means of duplicates. Duplicates of dissolved aluminium were within 37% (E horizons) or 6% (all other horizons) of the mean; duplicates for the depletion of the $Na_4P_2O_7$-extractable fraction were within 38% (E horizons) or 22% (other horizons) of the mean. Note that in the Hubbard Brook Bhs horizon KCl-extractable aluminium increases slightly over the course of 25 leaching cycles. This increase is due to the strong increase in dissolved aluminium relative to that in field conditions[11].

The cumulative amount of aluminium dissolved by HCl is invariably close to the amount of extractable ('free') aluminium removed (Fig. 1). Our data indicate that the dissolution rate of 'free' aluminium is high relative to that of the more abundant aluminosilicates (Table 1). Organically complexed ($Na_4P_2O_4$-extractable) aluminium is the fraction that dissolves most, whereas the exchangeable fraction (KCl-extractable) contributes little to the total dissolved aluminium (Fig. 1). Thus, aluminium is transferred to solution mainly from the organically complexed solid phase, even when the pool of organic aluminium is small.

On addition of HCl, dissolved aluminium concentrations invariably rose to constant values within 24 hours, indicating attainment of chemical equilibrium. Charge balances of the leachates indicate that dissolved aluminium is present mainly as aquo-Al^{3+}. Only in the leachates of the podzol Bhs horizons is a significant fraction (20–30%) of dissolved aluminium organically complexed. (The podzol Bhs is a soil layer rich in organic-aluminium precipitates.) Dissolved aluminium concentrations are significantly correlated with the organic ($Na_4P_2O_7$-extractable) aluminium content of the soil sample (Fig. 2). This further indicates the importance of solid alumino-organics as a major source of dissolved aluminium. Because dissolved aluminium concentrations in our leachates increased rapidly to stable levels, but were highly undersaturated with respect to gibbsite ($pAl-3pH=-8.11$; ref. 13 and Fig. 3), exchange equilibrium between dissolved and adsorbed aluminium as described for forest-floor samples[12] may also hold for solid-phase alumino-organics in our mineral soils.

Following Cronan *et al.*[12], the exchange equilibrium $3H^+ + AlX = H_3X + Al^{3+}$ can be modelled as $[Al^{3+}]/[H^+]^3 = K_{ex}(AlX/H_3X)^n = K_{app}$, where n, K_{ex} (exchangeable) and K_{app} (apparent) are constants, square brackets denote activities of aqueous ions and AlX and H_3X are their mole fractions on the organic sorbent. Taking negative logarithms, this expression becomes

$$pAl - 3pH = pK_{ex} - n\log(AlX/H_3X) = pK_{app}$$

The data in Fig. 3 indicate that pK_{app} increases with decreasing content of solid-phase alumino-organics. Only the first equilibrium extracts of the podzol Bhs horizons (represented by diamonds in Fig. 3) were close to equilibrium with gibbsite.

In general, the KCl-extractable aluminium fraction is assumed to represent readily mobilizable, exchangeable aluminium[14]. However, our data show that in a strongly acidic environment the $Na_4P_2O_7$-extractable aluminium fraction is a better estimate of readily mobilizable aluminium, exchanged against H^+.

In the field, 1–2% of organic aluminium is removed annually from the surface layers of the Dutch soils (Table 1). Note that the current rate of mobilization of aluminium from the uppermost soil layers is lowest where the content of soil organic aluminium is lowest. Based on the content of soil organic aluminium, the mobilization rates of aluminium are potentially high in the spodic B horizons. However, the availability of readily mobilizable aluminium is associated with a rapid acid consumption and consequently an increase in solution pH, which restricts further aluminium mobilization. Soil solutions in Bhs horizons are generally close to equilibrium with gibbsite. In the Dutch podzols the current depletion of alumino-organics is most pronounced in the upper few centimetres of the Bhs horizon, which was formed by precipitation of organic aluminium in the course of soil development. In such soils the

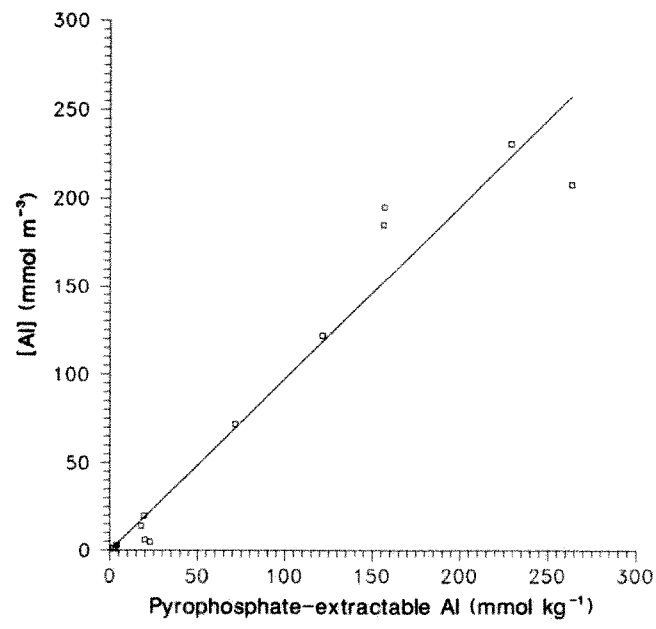


Fig. 2 Equilibrium aluminium concentrations in individual HCl leachates, after 10, 25 and 50 acid additions, as a function of $Na_4P_2O_7$-extractable aluminium. Data refer to all six soil horizons. Equilibrium concentrations of aluminium ([Al]) are significantly correlated ($R^2=0.9466$) with $Na_4P_2O_7$-extractable soil aluminium (Al_{pyro}): $[Al] = -0.60 + 0.98 \times Al_{pyro}$, where [Al] is in mmol m^{-3} and Al_{pyro} is in mmol kg^{-1}.

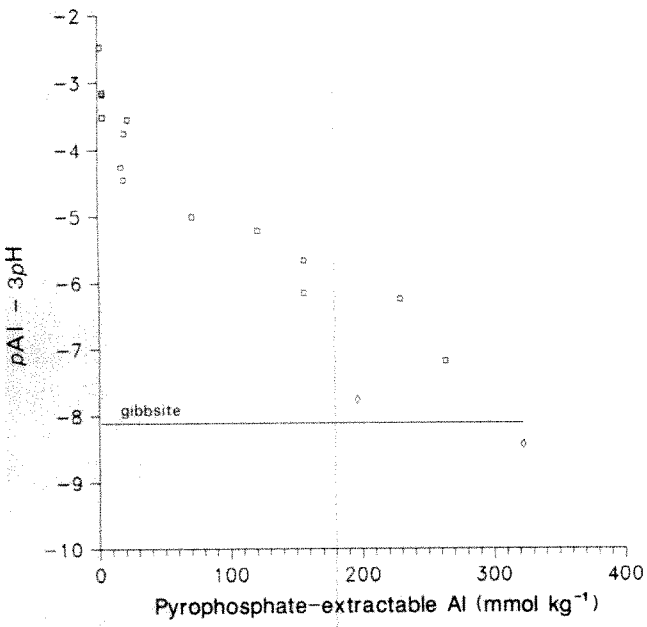


Fig. 3 Values for $p\mathrm{Al} - 3p\mathrm{H}$ in equilibrium leachates as a function of $Na_4P_2O_7$-extractable aluminium. Leachates are from all soil samples after 10, 25 and 50 HCl additions (squares). For the samples of the two podzol Bhs horizons values of the first leachate are also given (diamonds). Aluminium activities were calculated using the chemical equilibrium program ALCHEMI (ref. 18). For gibbsite we used $pK = -8.11$ (ref. 13).

introduction of acid rain produces a drastic change in the naturally occurring podzolization process.

The mobility of aluminium in the Hubbard Brook soils is significantly lower than in the Dutch soils (Table 1), because of higher soil-solution *p*H values, which is due, in turn, to a lower acid load and higher mobilization rates of base cations[15].

The current rapid depletion of organic aluminium in the rooting zone of many acid, sandy soils is irreversible on a timescale of decades or centuries. Because aqueous aluminium concentrations are in equilibrium with solid-phase alumino-organics, depletion upon continued acid deposition will inevitably lead to a reduction in dissolved aluminium concentrations. At the same time, soil-solution *p*H values, which are currently buffered in the *p*H range 3.5–4.2 by dissolution of aluminium[3] (J.M. *et al.*, preprint), may drop to values just below 3. Such dramatic changes in the soil solution chemistry of the rooting zone may have significant ecological consequences. Therefore, depletion of solid-phase organic aluminium and the associated changes in the chemical composition of soil solutions should be incorporated in current soil-acidification models.

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A new picture of the Moho under the western Alps

ECORS–CROP Deep Seismic Sounding Group*

The Moho—the boundary between crust and mantle first discovered by A. Mohorovicic[1]—is the main seismic marker in the continental lithosphere. The seismic nature of this interface, in terms of its position, topography, smoothness and continuity, may preserve imprints of the regional geodynamic evolution of the lithosphere. Here we report the results of a wide-angle seismic profiling experiment across the western Alps, which allows us to draw a cross-section of the Moho across this mountain belt. A tight sampling of this deep reflector shows abrupt changes in its depth and dip. The root zone of the chain (the zone of maximum crustal thickness) is well defined, with a 55-km-deep crust–mantle boundary. The Moho under the western Po plain is also clearly seen, and a shallow reflective structure is mapped under the Briançonnais zone in the 25–30 km depth range. This structure, if interpreted as lower-crustal or upper-mantle material, would support the hypothesis of a flaking of the lithosphere under the western Alps[2,3].

To prepare the layout of the vertical reflection line through the western Alps that formed the basis of the ECORS–CROP project[4,5] in 1986, a preliminary series of experiments[6] was conducted which aimed at mapping very deep interfaces along a cross-section extending from Grenoble (France) to the Po Plain (Italy). One-ton charges were detonated at five places (Fig. 1) and part of the recording array—low-frequency geophones and autonomous recorders—was deployed along fan profiles, a method which had already proved successful in other orogenic belts[7–9]. The fan radii were chosen so as to coincide with the theoretical maximum amplitude of reflections, corresponding to total reflection, at and beyond a critical distance. The Moho depth was assumed to be ~40 km, with an increase from west to east; the fan radii therefore ranged from 90 to 130 km. Because the velocity is assumed to be constant within the crust, and because each shot-station line is along the local strike of the Alps, no apparent dip of the reflector is expected. Each mirror point (triangles in Fig. 1) can therefore be plotted half-way between the shot-point and the station. With an average station spacing of 4 km along the fans, the reflectors are thus sampled every 2 km.

The fan data were processed to construct two composite cross-sections of the Alpine chain, showing the topography of deep reflectors (Figs 2 and 3). Figure 2*a*, corresponding to shots A, B and C, begins 30 km west of the Belledonne External Crystalline Massif (ECM) and extends through the Briançonnais and Piedmontese zones to the east of the Dora Maira Internal Crystalline Massif (ICM); Fig. 2*b* (shots D and LW) extends from the Gran Paradiso ICM to the Po Plain, intersecting the Sesia–Lanzo zone and the Canavese line, which marks the western limit of the chain. It should be understood that these

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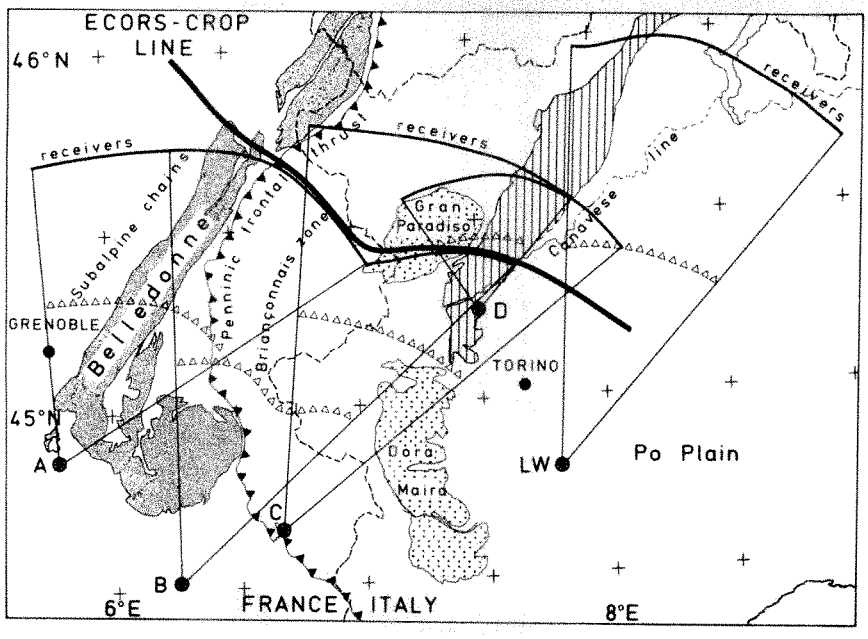


Fig. 1 Position map of the fan layout, showing the 5 shot-points A, B, C, D and LW. Open triangles indicate reflection points used to construct Fig. 2. Shaded area represents external crystalline massifs; dotted area represents internal crystalline massifs; hatched area represents the Sesia-Lanzo unit.

cross-sections combine fans with unavoidable offsets between them; for example, in Fig. 1, mirror points for shot D are shifted northwards by about 50 km relative to those for shot B. For this reason we chose to separate the two northern fans (D and LW) from the three southern ones, to keep a minimum of continuity within a composite cross-section. According to the Bouguer gravity map[10], the deep structures can be assumed to retain a cylindrical geometry for at least the 30-km extension across the section considered here.

On the seismic sections, reflections are picked using a maximum-amplitude criterion together with a correlation between traces. Around the critical distance, the wavelet reflected from the Moho is known to be very energetic, so that it can be identified with the maximum-amplitude signal. The sharpness of the onset of the reflected wavelet varies throughout the section (compare, for example, the two ends of the section in Fig. 2*a*), probably because of a variation in the seismic response of the reflector. Large (~20-km) variations in depth of the Moho marker are evident, and dips reaching 20° and extending over distances of tens of kilometres are observed. In the ECM area (Figs 2*a* and 3), the changes in depth and dip do not occur smoothly, as if by continuous flexure: from being almost horizontal at a depth of 37 km under the sub-alpine massifs and the ECMs, the Moho dips abruptly from 37 km to 55 km at their eastern edge, where the present-day maximum surface relief is found. Towards the innermost parts, under the Piedmontese zone and the Dora Maira ICM, it remains at a more or less constant, deep level of 55 km. The conversion of reflection times to depths depends on the mean crustal velocity, which is here taken to have the value 6.25 km s^{-1}; from previous seismic experiments[11-15], this value is known to be rather constant over this region so that we can assume that the depths thus obtained are accurate to better than 5%.

This deep Moho is overlaid by another seismic marker with high reflectivity (Fig. 2*a*, shot C). The maximum amplitude of the signals from the latter occur at depths of between 25 and 30 km, although it is difficult to determine accurate arrival times for these. Common experience in deep seismic profiling suggests that this reflective zone should be associated with a velocity contrast of more than 1 km s^{-1}, so that it corresponds either to the top of lower-crustal material or to a crust-mantle boundary. Situated at shallow depth and extending westwards beneath the Piedmontese zone and the ICMs as far as the Briançonnais zone, this reflector can be detected only when shooting from C. It acts as a mask which prevents the seismic energy from penetrating deeper, so that from shot-point C no deep Moho

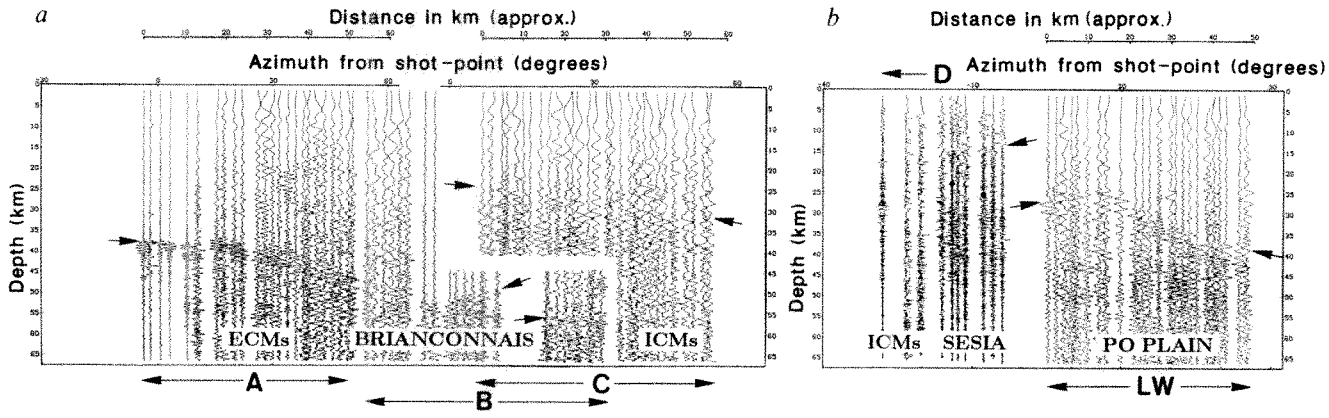


Fig. 2 Two composite cross-sections of the western Alps. *a*, The three fan profiles from shot-points A, B and C (see position on Fig. 1) are combined as a single record-section extending from the Chartreuse sub-alpine massif (far left) to the Dora Maira massif (far right). *b*, This shorter section combines results from shot-points D and LW and extends from the Gran Paradiso massif (far left) to the Po plain (far right). In each trace, the maximum-amplitude signal corresponds to reflection from the Moho. Earlier (shallower) energy arrivals may be ascribed to intracrustal discontinuities. For shot D, recorded at the relatively short distance of 40 km, the late (deep) arrivals are principally transverse S waves rather than reflected waves. Depths are calculated assuming a mean crustal velocity of 6.25 km s^{-1}.

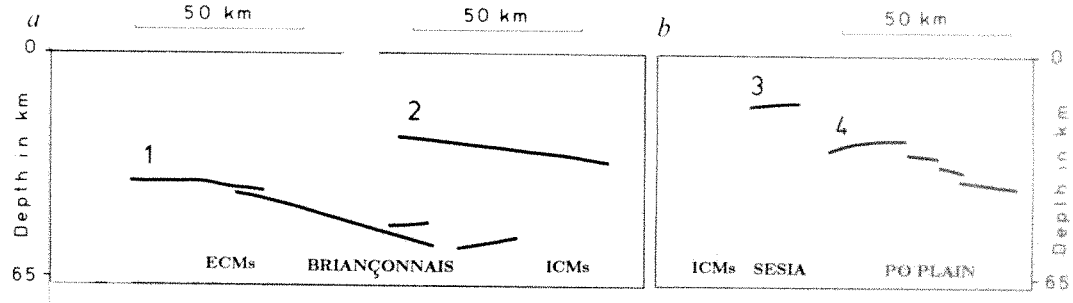


Fig. 3 Schematic line drawings of the profiles revealed by the corresponding seismograms in Fig. 2. (1) Autochthonous deep Moho; (2) Briançonnais reflector; (3) Ivrea body reflector; (4) hinterland (Po plain) Moho. In lithospheric flaking optics, features (2) and (3) trace the imbrication of anomalous upper mantle within the Alpine pile.

could be mapped. From shot-points A or B, however, the deep Moho can be reached, illustrating both the limited extent of this shallow unit and the importance of the shot/recording-array geometry in detecting such a feature.

The hinterland Moho (Fig. 2*b*), more than 35 km deep under the Po plain, where thick Tertiary–Quaternary sequences occur, seems to rise in a stepwise fashion to less than 25 km approaching the Canavese fault zone. Across this zone, there exists a gap in the seismic data, so that one cannot rule out the existence of another uprising of material with high seismic velocities. The Sesia zone is underlain by a strong reflector at a depth of only 13 km, which may correspond to a crust–mantle boundary. However, other deep reflectors in the same area, seen along a complementary longitudinal profile through Sesia, cast doubt on whether the hinterland Moho extends this far west and at such shallow depth; in a zone close to the Lanzo massif, where slices of lower-crustal and upper-mantle material have been brought up to the surface, reflective levels might be rather discontinuous. Nevertheless, the simplest structural scheme is to link the 13-km deep reflector discovered under the Sesia zone to the Po plain Moho further east.

From this wide-angle reflection profiling therefore emerges the first detailed picture of the geometry of the Moho beneath the Alps, incorporating even the innermost zones. Previous experiments[11–15] sampled the Moho too sporadically so that only smooth variations could be mapped. Close-ups of the sections presented here show that such a continuous geometry is not entirely correct, as was previously suggested by a teleseismic prospecting of lithospheric contrasts beneath the Alps[16]. This conclusion results mainly from the high spatial resolution made available by the arrangement of the fans, which provided a 2-km sampling interval of reflectors along the cross-sections.

The shallow reflective level that we have discovered under the Briançonnais zone has never before been mapped, although recently published models of Alpine orogenesis[2] have suggested such a feature. The presence of lower-crustal or upper-mantle material would indicate a tectonic thickening, resulting from previously adjacent eastern lithospheric segments having been thrust onto the western segment (the alternative, internal deformation of the sole crust, is unlikely). This would suggest flaking of the European lithosphere under the western Alps, which is discussed more thoroughly elsewhere[5].

The Ivrea body, an anomalously shallow mantle unit discovered from gravity evidence in early geophysical experiments in the Alps[11], is sampled here from shot-point D, but our experiment is unable to define its exact geometry. It is much too shallow (13 km depth) to be connected directly to the Briançonnais reflector, 30 km under the ICMs. The anomalous upper mantle imbricated within the Alpine crust therefore consists of two distinct units, the Ivrea body and the new unit discovered here.

The fan layout of the experiment proved successful in detecting very deep reflections, and the ECORS–CROP vertical reflection line was able to provide information on upper- and mid-crustal reflectors. Uniting vertical reflection profiling with the more versatile wide-angle reflection method therefore provides the sort of complementary information required to determine the deep structure of such complex orogens as the Alpine chain.

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Testing the completeness of earthquake catalogues and the hypothesis of self-similarity

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Self-similarity of the earthquake process is consistent with the observed linear *b*-value relation log $(N) = a - bM$, where N is the number of earthquakes with magnitude M. Deviations from linearity are believed to be due to statistical fluctuations because of the scarcity of events at large M, or from incompleteness because of a detection threshold at small M. Above some magnitude level, all local events are detected, because they exceed the noise background on the seismogram. As magnitude decreases, however, events go undetected as the seismic signal approaches the noise background. Thus more of the smallest events should be logged at night than during the day, because the cultural noise sources and winds are diminished at night, resulting in presumably quieter seismograms. Here we use this day-to-night noise modulation to develop a completeness test for earthquake catalogues; catalogues that indicate no significant modulation are considered complete. We test three catalogues and show that the completeness magnitude can be different from the magnitude at which the *b*-value departs from linearity. In particular, catalogues that show significant deviations in linearity at small M but are otherwise complete, are at odds with the hypothesis of earthquake self-similarity.

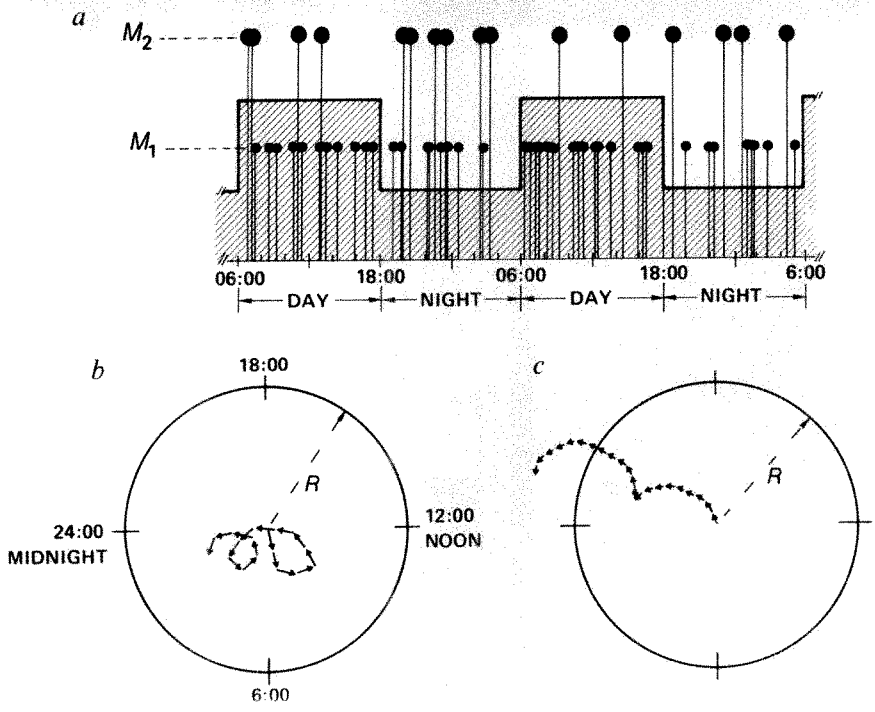


Fig. 1 *a*, An example of day-night noise modulation using a synthetic earthquake sequence of two different-magnitude events which follow Poisson distributions. The larger earthquakes (M_2) exceed the background noise (stippled area), so the phasor plot of these events (*b*) ($N = 16$, $R = 6.9$) shows no bias in the walkout. The smaller events (M_1) are detected only during the night, when the noise background is reduced, so these events show a significant phasor walkout (*c*) ($N = 50$, $R = 12.2$). The circles in *b* and *c* are drawn at the 95% confidence level for an equivalent random walk (Brownian motion). The Poisson rate for the smaller events is 1 event per hour and the *b*-value is 0.5.

Our method of testing for completeness follows one originally proposed by Schuster[1]. The test makes two assumptions about the earthquake process and the seismographic station, namely (1) that earthquakes, at any magnitude level, follow a Poisson distribution, and (2) that the daytime noise level is increased relative to night-time because of cultural activity and wind noise.

The randomly distributed (Poisson) nature of earthquake sequences (with aftershocks removed) is well documented in studies of seismic catalogues[2] but exceptions have been found, namely in a few cases that show modulations of the seismicity from tidal stresses of the Sun and the Moon[3,4], that is, through tidal triggering of earthquakes. A spectrum of the tidal forcing function, however, has negligible power at a period of exactly 24 hours[5], which is the period of the clock used in our test of completeness described below. The only known noise sources with a 24-hour periodicity are cultural and solar thermal effects (such as surface temperatures and winds); thus any daily modulations in the seismicity must be due to these sources. When observed, a modulation implies that some events at the detection threshold are missed during the half-cycle of increased noise, resulting in an incomplete catalogue. Our method of analysis, but with clocks tuned instead to the major tidal components, has been used successfully in studies of tidal triggering of earthquakes[3]. We have ignored the remote possibility of thermally triggered earthquakes, but note that the phase in Fig. 1 could be diagnostic.

Within a given magnitude level, the origin time of a seismic event is used to form a phase (or hour) angle on the local 24-hour clock; thus the catalogue of earthquake times is mapped into a set of earthquake phases. Each phase angle is assigned unit amplitude and then a phasor sum is performed over the set of individual unit phasors. The resulting phasor sum or 'walkout', R, is compared with that expected from a random process. For a set of random phases (that is, earthquakes that follow a Poisson process) the probability of obtaining a walkout larger than R is given by $P_R = \exp(-R^2/N)$. We reject the null hypothesis of randomly distributed earthquake phases when P_R is below some confidence level $(1 - P_R)$. Therefore, if the observed R exceeds some critical radius (a reasonable value is $1.73\sqrt{N}$, corresponding to a 95% confidence level), then the catalogue contains a significant bias in the day-to-night recording of earthquakes in the given magnitude interval and should be classified as incomplete at (and below) this magnitude. The sensitivity of our test is dependent on the number of events. For example, we can detect a 3% incompleteness (worst case) in a 10,000-event catalogue, increasing to 9% incompleteness for 1,000 events.

A simple example of day-night noise modulation is shown in Fig. 1. In this example, all the larger-magnitude earthquakes would be detected as they are clearly above the noise at any time of the day. The phasor walkout formed from this set of earthquakes grows as $\sqrt{N}$ and resembles Brownian motion because the phase angles are random, having been determined from earthquakes that have a Poisson distribution. The smaller-magnitude earthquakes are also Poisson-distributed but because more are detected when the noise is reduced (that is, during local night) they tend to form a non-random pattern with average alignment biased towards night-time hours. In this example, our test indicates that the larger-magnitude events are completely catalogued but the smaller ones are not. Of course, variations in noise levels in real catalogues are never as clear-cut as shown in our example, but the persistent bias introduced by any day-night noise modulation should eventually be detectable in catalogues of longer duration. As soon as the modulation becomes significant, the catalogue is shown to be incomplete. Intrinsic in the test is the confirmation that the modulation exists, because it becomes visible at lower magnitudes. The test provides an objective answer to the question of completeness, makes no assumptions about the linearity of the *b*-value curve, and allows one to set a confidence level on catalogue reliability to answer further questions regarding the underlying nature of the earthquake process.

Other problems arising in catalogues are the result of bursts of earthquake activity, such as swarms and aftershocks, which produce sections of highly non-random, and in worst cases almost linear, phasor walkout. There are algorithms for identifying (and removing) aftershocks[2], but we choose simply to ignore all shocks occurring more frequently than at twice the average hourly earthquake rate for the catalogue (again within a given magnitude interval).

We demonstrate our completeness test with catalogues of crustal earthquakes (0–30 km deep) obtained from three

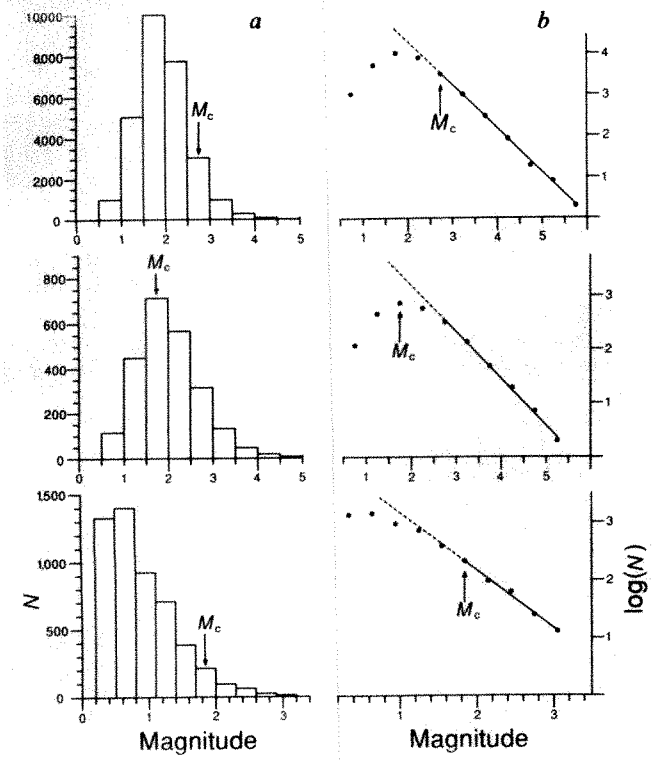


Fig. 2 Plots of *a*, the number of events N, and *b*, $\log(N)$, within given magnitude intervals for catalogues HAW (*a*), KMU (*b*) and POZ (*c*). The *b*-values are, respectively, 1.06, 0.87 and 1.00, and were determined using the points fitted with the solid straight line; the dashed line is an extrapolation using $\log(N) = a - bM$. Arrows mark the smallest magnitude at which a catalogue is complete, based on the phasor walkouts in Fig. 3.

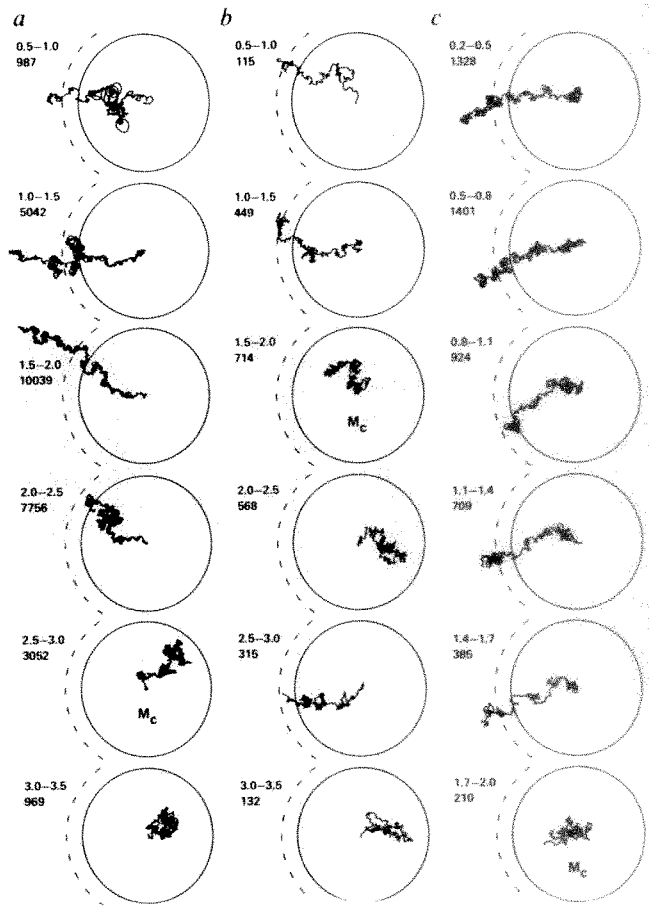


Fig. 3 Phasor sum plots for the catalogues HAW (*a*), KMU (*b*) and POZ (*c*). The numbers in each frame are the magnitude intervals and the numbers of events. The plots are time-oriented as in Fig. 1*b*, and scaled such that the confidence levels are drawn with constant radii (solid = 95%; dashed = 99% confidence level). A catalogue whose walkout does not span a chosen confidence level is considered complete at that level; thus M_C is the smallest magnitude of completeness (95%) for each catalogue in this figure.

different seismic arrays: (HAW) Island of Hawaii, coverage of ~80 km radius, 41 stations, 1977–1985; (KMU) southeast corner of Hokkaido, Japan, ~50 km radius, ~9 stations, 1976–1987; and (POZ) Phlegraean Fields near Pozzuoli, Italy, ~3 km radius, 22 stations, 1983–1984. Plots of the number of events versus magnitude are shown in Fig. 2 for these catalogues. The difference in the peak position and shape of the curves between the larger arrays HAW and KMU, and the smaller, concentrated array POZ is readily apparent (Fig. 2*a*), but *b*-values are still near unity (Fig. 2*b*), in general agreement with the concept of self-similarity. Notice also that catalogue POZ is divided into smaller magnitude intervals because of the increased sensitivity but limited-magnitude bandwidth of this array. The phasor walkouts as a function of magnitude for the three catalogues are shown in Fig. 3 for the same magnitude intervals used to construct Fig. 2. The smallest magnitude interval at which each of the catalogues passes the completeness test (95%) is labelled as M_C. We are confident that the data set at (and above) M_C is complete but we make no claims about the degree of incompleteness of the data below this point.

Catalogue HAW shows a departure from linearity in the *b*-value plot at ~1/2 magnitude below M_C. We assume that this non-linearity is caused by the loss of events below the threshold, rather than having the same cause as the violation of the linear relation seen for larger magnitudes.

In POZ, an obvious non-linear departure starts at ~1 magnitude below M_C and rolls off rather gradually. This catalogue is collected from a relatively small region compared with KMU and HAW and is therefore susceptible to very localized noise sources such as commuter traffic on a road during certain times of the day. Although this selective increase in background noise may have a small effect on the *b*-value curve, say a ~10% deficit, it is certainly within the range of detectability by our completeness test. POZ exemplifies a catalogue that does not show significant departures from linearity even though it fails the completeness test. In fact, when the number of events peaks, our test attains maximum sensitivity, in contrast to a *b*-value plot which has minimum sensitivity at peak N.

The KMU catalogue shows a highly significant fall-off in the number of smaller events even though the catalogue is apparently complete. Taylor and coworkers[6,7] reached the same conclusion in a recent study of this catalogue. We believe that this points to an important deviation in the common lore of linear *b*-value curves and the idea of the self-similar earthquake process.

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Distribution of seamounts in the North Atlantic

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The paucity of multi-narrow-beam bathymetric data in the ocean basins has meant that previous studies[1–8] of seamount distribution and size have relied on wide-beam echo sounder data and small amounts of Seabeam multi-narrow-beam data. Since 1967, however, the US Naval Oceanographic Office has been collecting multi-narrow-beam bathymetric data[9–14]. Here we use an extensive collection of such data to locate over 800 seamounts in the North Atlantic. We find that ridge crest-generated seamounts are formed only between Iceland and the Hayes fracture zone, where there are contributions of plume material to the mid-ocean ridge plumbing system. It appears that in the slow-spreading North Atlantic an injection of plume material into the ridge plumbing system is necessary for the formation of seamounts at the ridge crest.

We use the term seamount for all circular or elliptical features of volcanic origin, regardless of size, including those where flanking rift zones or slumping alter the basic circular or elliptical shape. The multi-beam data we studied are contoured at 10-fathom intervals (1 fathom = 6 feet). In some areas, such as the rift valley of the northern Reykjanes ridge[15], we were able to locate seamounts that had a relief of the order of 20–30 fathoms. Because of the rough block-faulted nature of the ridge, however, it is impossible, on the ridge flanks, to separate seamounts this small from the general ridge-flank tectonic fabric. To be certain that we counted only volcanic features, we have limited our study to seamounts that have a relief greater than 50 fathoms. Although there are undoubtedly smaller volcanoes that we did not count, the smaller seamounts, where detectable, have the same general distribution as those higher than 50 fathoms.

Figure 1 shows the location of seamounts in the North Atlantic with a relief greater than 50 fathoms. Excluding the Lesser Antilles, there are 810 seamounts. The blank areas reflect the lack of seamounts; the general distribution pattern is unlikely to be affected by the lack of data. We estimate that the total number of seamounts with heights greater than 50 fathoms is unlikely to be underestimated by more than 10% as a result of incomplete data coverage. Sediment thickness in the North Atlantic is highly variable[16] and it is likely that smaller volcanoes have been buried. For example, in the area between the New England and Corner seamounts there are a number of small seamounts that are probably the tops of larger seamounts buried by sediments from the Sohm abyssal plain. The pattern of sediment distribution in the North Atlantic does not, however, correspond to the distribution of seamounts shown in Fig. 1, indicating that the general seamount distribution pattern is unlikely to be affected by sediment burial.

Morgan[17,18] and Duncan[19] have described the traces of the major hotspots in the North Atlantic. Figure 1 shows the distribution of seamounts in these traces, in addition to several smaller traces. Madeira and Canary hotspots generated the Milne and Newfoundland seamount groups, respectively, before crossing to the east side of the Mid-Atlantic Ridge (MAR). The Canary hotspot is probably responsible for the cluster of seamounts located at 38°–41° N and 15°–18° W. Both of these hotspots passed through the Madeira–Tore Rise and Horseshoe seamount areas[19]. In addition, the Africa/Eurasia plate boundary passes through the Horseshoe seamount area, so it is not clear which of the seamounts in this area were generated directly by the hotspots and which might be due to hotspot interaction with the plate boundary. The trace of the Azores hotspot is uncertain. According to Morgan[17] the hotspot would have been near Coruna 'seamount' at about 60 Myr ago and would have followed the Azores–Biscay rise to its present position. (Coruna is located at 44° N 14° W, but it does not appear to be volcanic and is not plotted in Fig. 1.) The trace was undoubtedly affected by interactions with the MAR and the Iberia–Africa/Eurasia plate boundary. Great Meteor hotspot generated the New England and Corner seamounts before crossing the MAR and generating the seamounts in the vicinity of Cruiser and Great Meteor. The hotspot was near the ridge crest during the formation of the Corner seamounts, ~75 Myr ago, and remained near the crest until ~38 Myr ago, when it crossed to the east side of the ridge between the Oceanographer and Hayes fracture zones[19]. The group of seamounts between the New England seamounts and Bermuda can be attributed to Verde hotspot[19], but is is not clear if the three small groups located along 26–27° N between 20° and 28° W are also due to Verde. Also, the origin of the three clusters west of the Cape Verde islands is unclear. The Researcher seamounts and the Barracuda/Researcher ridge form chains of volcanoes that are co-polar with the other hotspot traces in the western North Atlantic and are thus probably due to weak hotspots. The North Brazilian ridge is a line of seamounts in the Ceara abyssal plain that also may be a hotspot trail and may be related to either the Bathymetrists or Sierra Leone hotspots. The Charcot 'seamounts', located along 45° N between 10 and 15° W, do not appear to be volcanic in origin and are probably an expression of the extinct Iberia–Africa/Eurasia plate boundary.

The interaction of hotspots with mid-ocean ridge (MOR) systems has been discussed extensively[20–26]. Morgan[27] suggested that the hotspot did not have to be directly under the MOR for this interaction to occur; conduits for the flow of plume material to the MOR plumbing systems could be maintained as the MOR moved away from the hotspot. Schilling[28,29] has elaborated on this model and, on the basis of extensive sampling of the crest of the MAR, proposed that along-ridge geochemical gradients about ridge-centred hotspots can be attributed to incompatible-element-rich magmas which rise as plumes or blobs beneath the hotspots and mix with the incompatible-element-depleted asthenosphere that normally feeds the spreading ridge. In the North Atlantic, Schilling[29] identifies hotspot contributions from Jan Mayen, Iceland, 45° N, Azores, 35° N and 14° N. The K_2O, La/Ti and La/Sm anomalies associated with Iceland extend south of Iceland to about 60° N. The strontium anomaly reaches a minimum at 60° and appears to increase toward the Charlie–Gibbs fracture zone. The Iceland helium anomaly, however, extends south to the Charlie–Gibbs fracture zone (see Schilling[29], Fig. 3 and Plate 8B). There is a narrow geochemical anomaly associated with the Charlie–Gibbs fracture zone. Between the Charlie–Gibbs and Kurchatov fracture zones there is a broad geochemical anomaly centred at 45° N. There appears to be a narrower superimposed anomaly at 43° N. These anomalies cannot be attributed to known hotspots. If they are due to separate hotspots, then these hotspots did not generate seamount chains or other bathymetric expressions. The geochemical anomalies caused by the Azores hotspot extend between the Kurchatov and Hayes fracture zones. There is a small contribution at 35° N between the Pico/East Azores and Hayes fracture zones that probably comes from Great Meteor hotspot[30]. South of the Hayes fracture zone there is no plume component except for a small anomaly at 15° N, for which Barracuda/Researcher hotspot may be responsible.

There is a first-order correlation between the distribution of seamounts along the MAR and the hotspot contribution to MAR magmas. Seamounts are formed only between Iceland and the Hayes fracture zone where there are contributions of plume magma to the MAR plumbing system. South of the Hayes fracture zone there are no ridge crest-generated (RCG) seamounts and only the small geochemical anomaly at 14° N. The

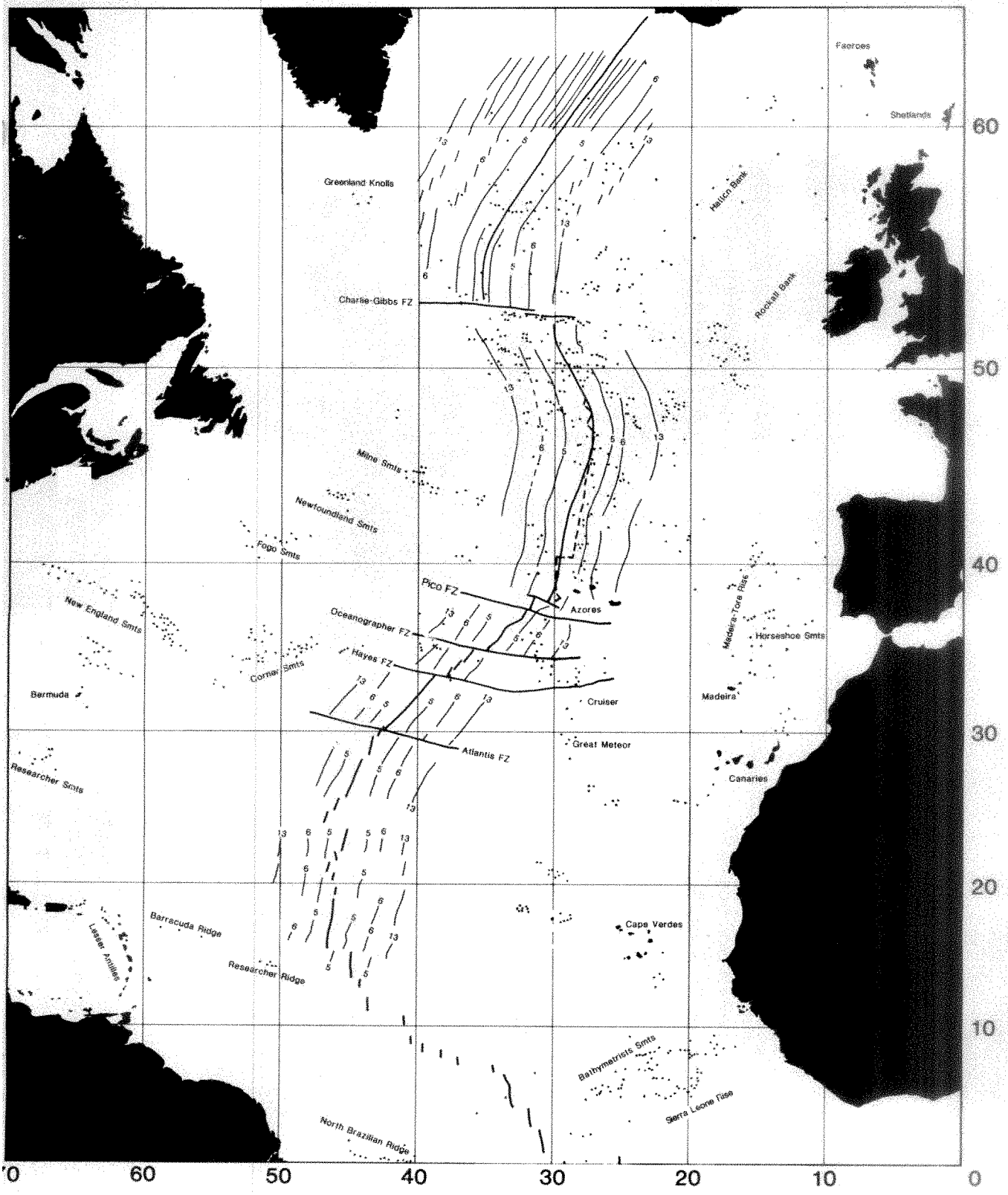


Fig. 1 The locations of seamounts in the North Atlantic with heights greater than 50 fathoms (shown as dots). Magnetic lineations are from S. Cande (personal communication). The dashed line shows the location of the topographic high between the Charlie–Gibbs fracture zone and the East Azores fracture zone; it does not correspond to anomaly zero.

atter is relatively recent[31] and it may be that the Barracuda/Researcher hotspot has contributed only enough material to the plumbing system to affect the geochemistry but not enough to generate seamounts. The detailed relationships between the distribution of seamounts and plume contributions to the MAR plumbing system are more complex. In addition to the variation in seamount numbers along the MAR, it is obvious from Fig. 1 that seamount generation varies with time and is not always symmetrical about the ridge axis. Figure 2 shows the number of seamounts for every one degree of latitude in 0–10, 10–20 and 20–35 Myr time windows (corresponding to magnetic anomalies 1, 5, 6 and 13) for the east and west sides of the

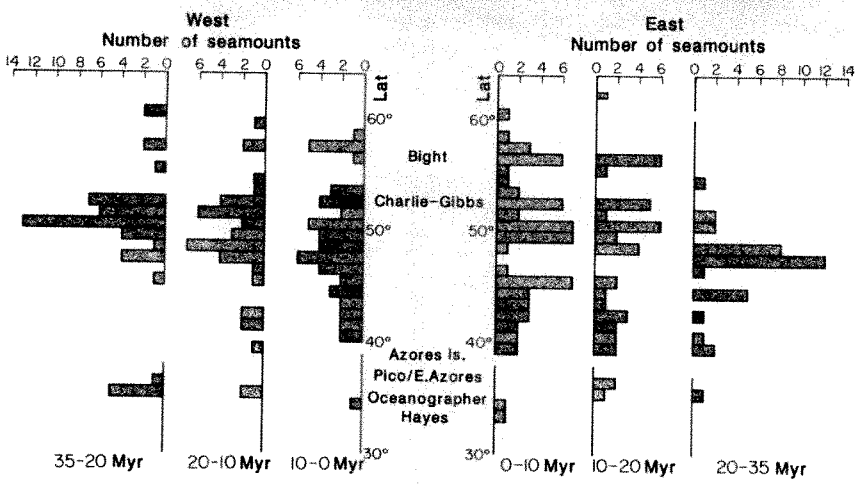


Fig. 2 The number of seamounts plotted against latitude in 0–10, 10–20 and 20–35 Myr time windows (corresponding to magnetic amomalies 1, 5, 6 and 13) for the east and west sides of the Mid-Atlantic Ridge. The latitudes of fracture zones and the Azores islands are indicated. Where the base line of the graphs is omitted there is insufficient data to provide an accurate count of seamount numbers.

MAR. The greatest concentration of seamounts occurs between the Charlie-Gibbs and the latitude of the Azores islands. This presumably reflects the strong contribution of plume material to this section of the MAR by the 45° N and Azores hotspots. The distribution of seamounts on the west side of the ridge suggests that these hotspots started contributing material to the plumbing systems at ~45 Myr in the section of ridge just south of the Charlie-Gibbs, and that the interaction is no older than ~20 Myr at the present latitude of the Azores. On the east side of the ridge the seamount distribution indicates an older and more complex interaction. There is a significant cluster of seamounts on crust older than anomaly 13 between 47° and 49° N, an absence of seamounts between the ridge crest and anomaly 6 at 46° to about 49° N, and a large cluster just west of the Ireland continental shelf at 49° to 52° N. This complexity in seamount distribution is most probably related to the reorganization in plate boundaries in this area and the interaction between the ridge plumbing system and the hotspots. Between ~60 and 35 Myr, King's trough, the Azores-Biscay rise and the Charcot 'seamounts' formed the plate boundary between the Iberia-Africa and Eurasia plates[32,33]. The Azores hotspot was moving along the Azores-Biscay rise during this time[17]; however, the location (or existence) of the 45° N hotspot during this time is uncertain as it did not leave a bathymetric trace.

The seamounts between the Azores and the Hayes fracture zones may be due to the plume contribution from Azores hotspot or from Great Meteor hotspot. Morgan[18] suggested that the group of seamounts between Cruiser and Great Meteor were formed directly by Great Meteor hotspot and that the cluster of seamounts between Cruiser and the Oceanographer fracture zone was formed by the interaction of the hotspot with the MAR. Great Meteor hotspot is nearly 900 km from this section of ridge crest[28], so its contribution to the MAR plumbing system is probably small. The geochemical anomaly associated with the Azores, on the other hand, extends past the Pico/East Azores fracture zone to the Hayes. The offset across the Pico/East Azores fracture zone decreases significantly between anomalies 6 and 5. In the vicinity of the Azores the ridge crest appears to be 'pulled' toward the Azores (as indicated by the dashed line in Fig. 1), and although there is a distinct bathymetric expression of the fracture zone east and west of the ridge crest, there is no distinct bathymetric offset of the present ridge crest. Thus, there may be no blockage of flow at the Pico/East Azores fracture zone. On the other hand, the offset on the Oceanographer fracture zone is 120 km and the offset on the Hayes is 80 km[34]. It is not clear why material flows past the Oceanographer but not past the Hayes, unless the volume of flow is nearly depleted by the time it gets to the Hayes.

With the exception of the helium anomaly, and perhaps the strontium anomaly, the geochemical anomalies associated with Iceland hotspot do not extend south of 60° N[29]. There i however, no offset in the MAR at this latitude that would indica a blockage of flow. There are very few seamounts along th Reykjanes ridge between Iceland and 60° N whereas there ar a significant number between 60° N and the Charlie-Gibb fracture zone. Seamounts have been generated along this sectio of the MAR for the past 35 Myr, although the seamounts aroun the periphery of Rockall bank and the cluster between th southwest corner of Rockall and the Charlie-Gibbs may hav been formed at the ridge crest before 35 Myr. The section fror 60° N to Charlie-Gibbs is the one section of the MAR wher seamounts are formed without a strong hotspot signal in th geochemistry.

The line of seamounts along the Bight fracture zone (show in Fig. 1 as an east-west line of seamounts at 57° N) is responsibl for the peak in the 0-10 Myr and 10-20 Myr windows in Fig. 2 It seems likely that the fracture zone provided easy conduits fo these volcanoes[2,7], although it is obvious that fracture zones ar not necessary for seamount formation in the North Atlantic There must be local perturbations to the plumbing system o other zones of weakness in the crust that control the locatio of the majority of the seamounts.

The first-order correlation of RCG seamounts with the geo chemical data indicates that an injection of plume material i necessary for the formation of RCG seamounts. This suggest that in a normal spreading situation the MAR plumbing syster has only enough pressure to lift magma to the top of the crust which follows if decompression melting is the only source o magma. An injection of plume material overpressurizes th plubing system; some of the excess pressure is released by th formation of seamounts, and some may be released by th formation of thick crust. The seamounts act like piezometers ir the plumbing system and allow an estimation of the minimum overpressure resulting from the plum contribution. Seamount with heights of ~1,500 fathoms are associated with the Charlie-Gibbs fracture zone, requiring an overpressure of ~42 MPa. Fo comparison, this is about half of the pressure in the Kilauea summit reservoir before the 1960 eruption on Kilauea's lowe east rift zone[35]. The North Atlantic is thought not to have a steady-state magma chamber, and Schilling[29] suggests that small discontinuous, transient magma chambers may be more likely in slow-spreading ridges. If so, the overpressure almost certainly varies with distance along the ridge and with time, although it is not clear how these variations are reflected in the distributio of seamounts. The formation of seamounts between 60° N and the Charlie-Gibbs could be explained if the Iceland plume contribution pressurizes the MAR plubing system as far south as the Charlie-Gibbs, but the plume material flows only as fa as 60° N. A similar phenomenon is observed in Kilauea, where pressure changes in the summit reservoir are propagated down

the east rift zone before magma moves along the rift zone.

The model presented above suggests that the processes responsible for the generation of seamounts on the MAR are relatively simple, although the detailed variations in plume contributions and seamount distribution suggest that factors such as the volume of plume material, conduit geometry, location and offset of fracture zones, and the timing of plate reorganizations and hotspot interactions must also be considered. The North Atlantic may constitute the slow-spreading endmember of these processes, and it is not clear how, or if, these processes are applicable to faster spreading ridges.

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Geochemical evidence for invasion of Kilauea's plumbing system by Mauna Loa magma

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From the beginning of the study of Hawaiian volcanism there has been controversy over possible relationships between the neighbouring active volcanoes Mauna Loa and Kilauea[1–5]. Seismic activity, thought to reflect upward migration of magma, reveals that the magmatic plumbing systems apparently converge at depth to form a broad funnel within the mantle[6]. Although on rare occasions they have erupted concurrently, the brief historical eruptive record appears to show that when Kilauea is most active, Mauna Loa is in repose and vice versa, suggesting that they may be competing for the same magma supply[5,7]. Petrological, geochemical and isotope data, however, require a diametrically opposite conclusion. Distinct differences in major-element, trace-element and isotope compositions of lavas are regarded as compelling evidence that the two volcanoes have separate magmatic plumbing systems, supplied by parental magmas from physically and geochemically distinct mantle sources[8–13]. Here we present preliminary geochemical data which show that in the past 2,000 years Kilauea has erupted a spectrum of lava compositions resembling historical Kilauea lavas at one end and Mauna Loa lavas at the other. We discuss the cause of this diversity, and speculate that magma from Mauna Loa may have invaded Kilauea's 'high-level' magmatic plumbing system.

Most surface lava flows on Kilauea are younger than 1 kyr (refs 14–16). To examine more of the recent prehistoric record, we sampled successive flows exposed in the walls of two pit craters, Kilauea Iki and Pauahi[17]. Kilauea Iki is close to the summit caldera; Pauahi is about 6 km down Kilauea's upper east rift zone. Age control for these flows depends in part on palaeomagnetic results[14,15], and in part on the age of the Uwekahuna Ash, a thin phreatomagmatic deposit derived from Kilauea's summit[4,15,18,19]. The Uwekahuna Ash is well exposed in the walls of Pauahi crater, about 45 m above the floor, but is not present in the walls of Kilauea Iki. From ^{14}C measurements, Lockwood (personal communication) infers an age of 2.1 kyr for the ash. Using this age and palaeomagnetic results[14,15], we infer that lavas exposed in the walls of Pauahi crater range in age from greater than 2.1 to ~0.4 kyr, and those in Kilauea Iki range from 1.5–2.0 kyr to ~0.4 kyr.

Compositional differences between historical Kilauea and Mauna Loa lavas, which have been thoroughly documented[8–13], are readily apparent from the ratios of Zr/Nb, Zr/Y and La/Yb (Figs 1 and 2), and from the averaged data summarized in Table 1. Previous studies propose that, at a given MgO content, prehistoric Kilauea lavas are more 'Mauna Loa-like' than their historical counterparts[9,20]. Disputing this conclusion, Easton and Garcia[21] found that Hilina Formation lavas older than 22 kyr exposed on Kilauea's south flank are compositionally close to modern lavas. Our data show that Kilauea lavas erupted over the past 2.0 kyr vary significantly in composition, particularly in abundances and ratios of the incompatible elements. In Fig. 1, historical lavas (AD 1823–1987) are characterized by Zr/Nb ratios of $\sim 10 \pm 1$, whereas prehistoric lavas are more varied and display a distinctly bimodal distribution with maxima at ~11 and 14. The lavas with Zr/Nb ratios of ~14 are similar in both major- and trace-element composition to Mauna Loa lavas (Figs 1 and 2; Table 1). Prehistoric Kilauea lavas, with lower Zr/Nb ratios, show a range of compositions from those broadly resembling modern Kilauea ($Zr/Nb < 11$) and Hilina Formation lavas, to others with compositions intermediate ($Zr/Nb = 11–12.5$) between those of historical Mauna Loa and Kilauea lavas (Table 1; Fig. 2).

In Pauahi crater, the Mauna Loa-like flows are restricted to a few thin flow units immediately above the Uwekahuna Ash, and close to the top of the section (Fig. 2). The remainder of the section comprises flows with compositions closer to, but not identical with, modern Kilauea lavas[17]. Flows on the southern wall of Kilauea Iki crater grade from Kilauea-like compositions near the base, through intermediate compositions, to Mauna Loa-like flows in the middle of the section, and back through intermediate compositions to Kilauea-like flows at the top (Fig. 2)[17].

To improve temporal control, we have also analysed samples of nine eruptive units from Kilauea's summit area, with ages

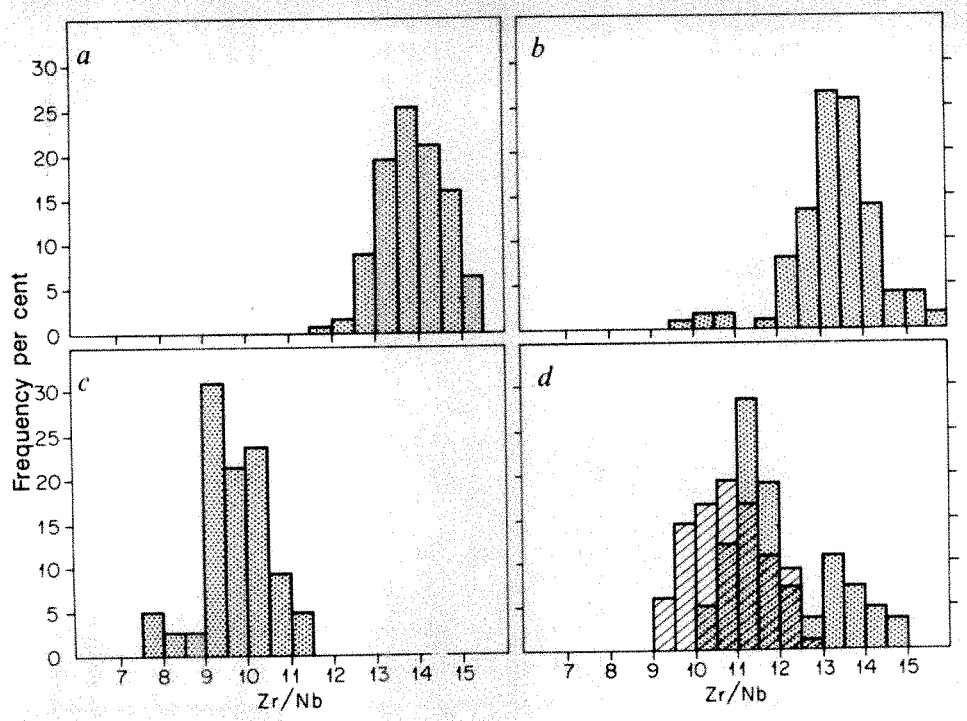


Fig. 1 Histograms of Zr/Nb ratios for historical (AD 1843–1984; $N = 137$) (*a*) and prehistoric (<30 kyr; $N = 130$) (*b*) Mauna Loa lavas, and historical (AD 1823–1987; $N = 42$) (*c*) and prehistoric (*d*) Kilauea lavas. Note the greater range and bimodal distribution for recent prehistoric (<2 kyr) as compared with historical Kilauea lavas. Hilina Formation lavas (≥22 kyr) (hatched bars in *d*; $N = 41$) have Zr/Nb ratios that are close to those of historical lavas[21].

Table 1 Comparison of average MgO-normalized major- and incompatible-trace-element abundances in prehistoric Kilauea lavas with their abundances in historical Kilauea and Mauna Loa lavas

	Historical Mauna Loa lavas		Mauna Loa-like		Prehistoric Kilauea lavas Intermediate		Kilauea-like		Historical Kilauea lavas	
Zr/Nb	(>12.5)		(>12.5)		(11.0–12.5)		(<11.0)		(<11.0)	
Number of samples*	122/28		20/7		46/10		11/3		30/9	
	X	s.d.	*X*	s.d.	*X*	s.d.	*X*	s.d.	*X*	s.d.
SiO_2	51.61	0.23	50.83	0.29	50.54	0.54	50.48	0.31	50.04	0.42
TiO_2	2.08	0.06	2.27	0.06	2.49	0.17	2.51	0.07	2.65	0.13
Al_2O_3	13.62	0.10	13.78	0.13	13.45	0.34	13.34	0.19	13.42	0.21
Fe_2O_3†	12.05	0.14	11.88	0.18	12.16	0.64	12.28	0.47	12.26	0.23
MnO	0.17	0.01	0.17	0.00	0.17	0.01	0.17	0.01	0.17	0.01
MgO	7.00	—	7.00	—	7.00	—	7.00	—	7.00	—
CaO	10.49	0.13	10.92	0.12	11.06	0.45	11.16	0.36	11.24	0.32
Na_2O	2.20	0.13	2.42	0.28	2.55	0.42	2.42	0.33	2.32	0.09
K_2O	0.39	0.04	0.40	0.02	0.44	0.05	0.46	0.02	0.51	0.04
P_2O_5	0.24	0.02	0.23	0.01	0.24	0.02	0.25	0.02	0.27	0.02
Total	99.84	—	99.89	—	100.11	—	100.06	—	99.88	—
Rb	6.0	0.7	6.0	0.4	7.2	0.9	7.6	0.9	9.4	2.1
Sr	313	21.3	330	9.1	337	16.0	349	11.9	372	29.0
Nb	9.8	0.7	10.5	0.6	13.3	1.0	14.7	0.8	17.8	1.9
Zr	134	7.0	142	4.4	154	9.1	157	7.7	173	10.5
Hf	3.3	0.13	3.5	0.1	3.8	0.2	—	—	4.8	0.5
La	9.3	0.9	10.2	0.9	11.1	0.6	12.7	0.3	15.9	1.7
Ce	25.1	1.9	27.3	2.6	29.4	1.7	34.7	1.7	39.4	3.6
Sm	4.8	0.2	5.2	0.5	5.5	0.4	5.9	0.3	6.3	0.3
Eu	1.70	0.05	1.82	0.08	1.84	0.06	1.92	0.14	2.08	0.16
Yb	2.05	0.06	2.03	0.13	1.99	0.07	1.99	0.17	2.11	0.17
Lu	0.28	0.01	0.29	0.02	0.29	0.02	0.29	0.02	0.30	0.03
Y	23.6	0.8	24.5	0.7	24.9	1.1	25.4	1.2	25.2	1.0
Zr/Nb	13.7	0.6	13.6	0.6	11.6	0.4	10.7	0.2	9.8	0.6
Zr/Y	5.7	0.2	5.8	0.1	6.2	0.2	6.2	0.3	6.7	1.3
K/Y	136	12.4	136	8.0	147	16.5	151	10.2	168	11.6
Sr/Nb	32.0	1.4	31.6	1.8	25.5	2.0	23.8	1.0	20.9	2.7
$(La/Yb)_{ch}$‡	2.7	0.3	3.0	0.2	3.4	0.3	3.9	0.4	4.6	0.4

The data have been normalized to a constant 7.0% MgO by addition or subtraction of olivine (Fo_{88}). Lavas that depart from an olivine-controll trend have been excluded from the compilation. Major- and trace-elements Rb, Sr, Nb, Zr and Y were determined by X-ray fluorescence (XR at the University of Massachusetts. Rare-earth elements and Hf were determined by instrumental neutron activation analysis (INAA) in the laborato of F. A. Frey at Massachusetts Institute of Technology. Data are from this study and refs 13, 17, 22 and 23. s.d., Standard deviation.

* Numbers refer to those analysed by XRF and INAA respectively.

† Total iron expressed as Fe_2O_3.

‡ Chondrite-normalized.

constrained by ^{14}C dating to between ~0.25 and 1.1 kyr. With the exception of the youngest flows (<0.4 kyr), which resemble modern Kilauea lavas in composition, all these flows show compositional variations similar to the pit crater flows. Figure 2 and Table 1 show very clearly that there is a continuum of compositions for prehistoric Kilauea lavas ranging from those that resemble modern Kilauea lavas to those that resemble historical Mauna Loa flows. More have intermediate compositions than have either Kilauea-like or Mauna Loa-like compositions. Figure 3 illustrates a possible evolutionary scenario for the distribution of lavas of varied composition in Kilauea's summit and upper east rift zone.

Most Mauna Loa lavas are restricted in composition, clustering at the intersection of an olivine-controlled trend with a trend for more differentiated lavas. Typically, they contain ~6.8–8.0% MgO (refs 22, 23). Rhodes[23] attributes this uniformity to eruption from a continuously replenished shallow magma reservoir. The Mauna Loa-like lavas on Kilauea also share these characteristics, with MgO content between 6.6 and 8.6%. In contrast, Kilauea-like and intermediate lavas are more variable, ranging from differentiated basalts (5.5% MgO) to picrites (21% MgO)[17].

The presence on Kilauea of flows with compositions that strongly resemble magma processed through Mauna Loa's shallow plumbing system might reflect flooding by Mauna Loa flows of the adjacent, and lower, Kilauea shield[15]. Such flows would have travelled over 20 km, originating on Mauna Loa's northeast rift zone. Far-travelled flows are typically dense tube-fed pahoehoe or thick aa units; the map relations, flow thickness, texture and vesicularity of these flows, on the other hand, indicate that they did not travel far, but were erupted on Kilauea. Furthermore, in our extensive investigations of Mauna Loa, no flows with intermediate compositional characteristics have been found throughout over 30 kyr of eruptive history[9,23].

This raises several intriguing possibilities. The magmas supplying Kilauea over the past 2 kyr may have been derived directly from the mantle either by partial melting of a mixed, multi-component mantle source, or by mixing of magmas from two or more distinct sources. Alternatively, they may have been supplied indirectly, as would be the case had magma from Mauna Loa invaded Kilauea's magmatic plumbing system.

The heterogeneous nature of the mantle source for Hawaiian volcanism is well established[11,13,24,25]. However, it is mainly during the evolutionary stages of a volcano's history[5], typically over time periods of a million years or more, that major temporal changes in lava composition occur. With the exceptions of Koolau[26] and Kahoolawe[27], the tholeiitic shield-building stages[5] of most individual Hawaiian volcanoes are characterized by relatively uniform and distinctive geochemical features[28]. Our unpublished data for Mauna Loa show that prehistoric lava compositions have remained comparatively homogeneous for over 30 kyr (Fig. 1). Similarly, on Kilauea, lavas of the Hilina Formation (>22.5 kyr) remained relatively uniform in composition over perhaps as long as 50 kyr (ref. 21). We suggest, therefore, that compositional fluctuations in the mantle source within a time frame of less than 2.0 kyr are unlikely.

Although partial melting of a heterogeneous mantle source cannot be ruled out, we are persuaded otherwise by two key observations. First, Mauna Loa-like lavas erupted on Kilauea have all the compositional attributes of lavas processed through Mauna Loa's plumbing system. Second, these and intermediate-composition lavas first appear stratigraphically above the Uwekahuna Ash. The Uwekahuna Ash is thought to have been produced by a major explosive eruption associated with caldera collapse, which was itself initiated by massive magma drainage along one of the two rift zones[4,15,18,19]. Evidence for extensive magma movement and drainage along Kilauea's east rift zone before the explosive 1924 summit eruption has been well documented[4,5]. The possible magnitude of this process is illustrated by the recent discovery of voluminous (2-km^3), relatively young

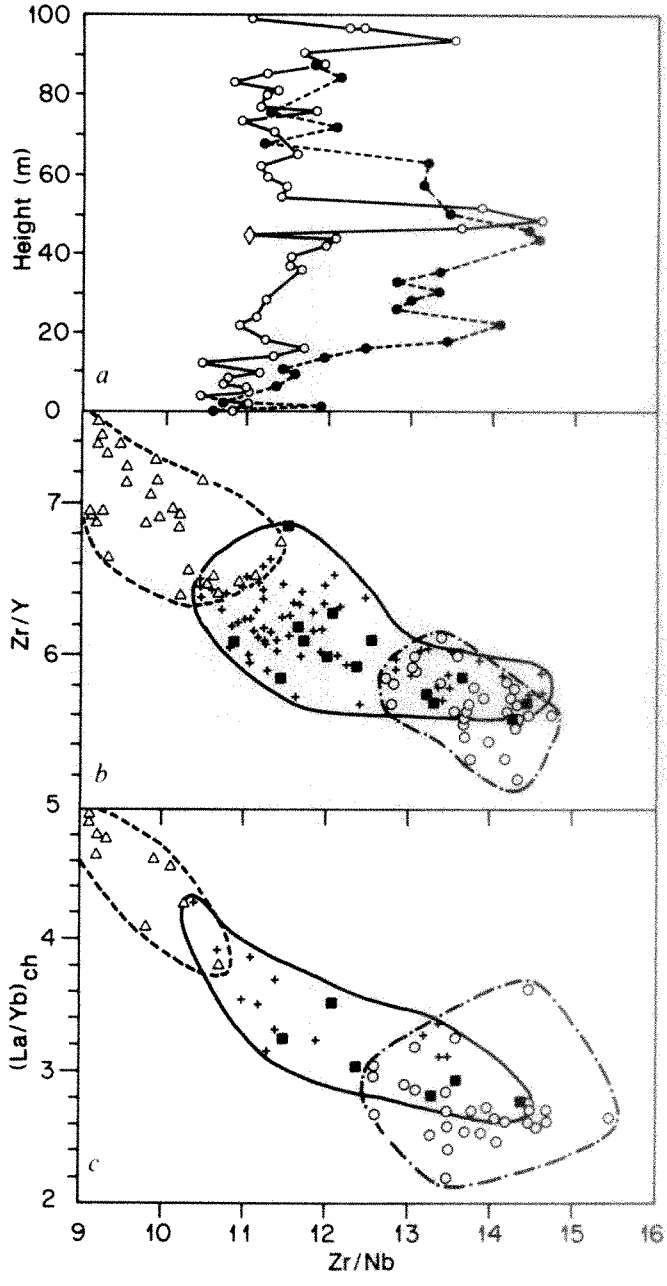


Fig. 2 *a*, Variation in Zr/Nb ratio with stratigraphic height in the walls of Pauahi (open circles joined by solid line) and Kilauea Iki (filled circles joined by dashed line) pit craters. Note the presence of the Uwekahuna Ash (open diamonds) at 45 m in the Pauahi section; this ash is presumed to be below the pit crater floor in the Kilauea Iki section. *b*, Zr/Y versus Zr/Nb ratios for Kilauea Iki and Mauna Loa lavas. Field for prehistoric Kilauea lavas shown as a solid line. ■ Prehistoric Kilauea lavas (200–1,100 yr BP) with ^{14}C age control; + Pauahi and Kilauea Iki lavas; △, Historical Kilauea lavas, field shown as dashed line; ○ Historical Mauna Loa lavas, field shown as dot-dashed line. *c*, Chondrite-normalized La/Yb versus Zr/Nb ratios for Kilauea and Mauna Loa lavas. Key is the same as for *b*. In *b* and *c* prehistoric Kilauea lavas reveal a range in composition from historical Kilauea to historical Mauna Loa.

lava flows emanating from the submarine extension of Kilauea's east rift zone, which are thought to represent such magma drainage[29].

We speculate that, following the voluminous magma drainage responsible for the Uwekahuna event, magma stored in the adjacent shallow plumbing system of Mauna Loa may have invaded Kilauea's evacuated plumbing system. Some of this

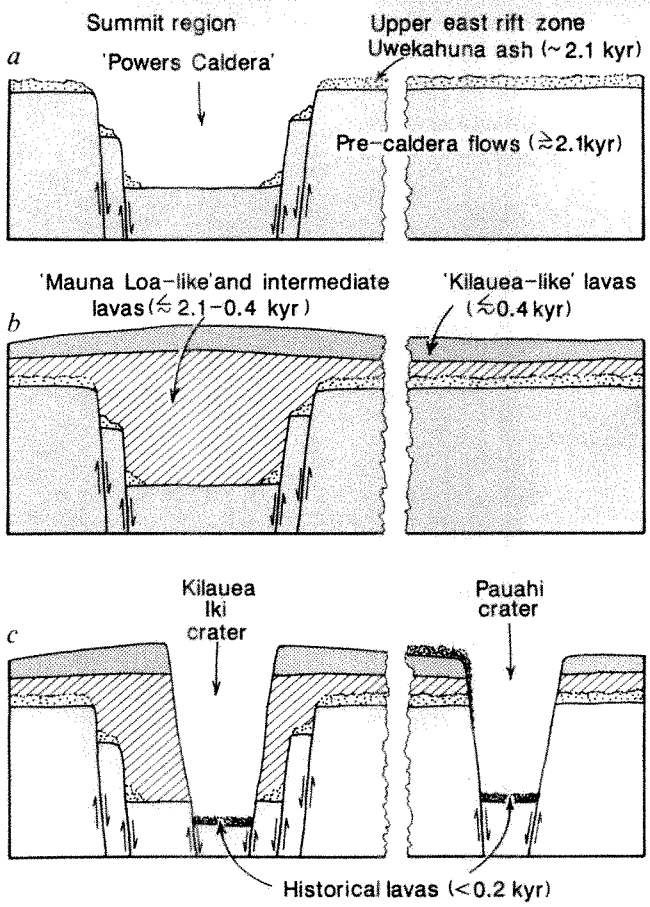


Fig. 3 Schematic representation of the inferred evolution of the geochemical variations in Kilauea summit and upper-east-rift-zone lavas over the past 2.1 kyr following the eruption of the Uwekahuna Ash. *a–c* represent successive configurations in time. The Uwekahuna Ash is a widespread phreatomagmatic base-surge deposit, derived from Kilauea's summit. It is thought to be associated with the collapse and formation of the ancestral 'Powers Caldera'[4,15,18,19]. Lavas erupted before the formation of the Uwekahuna Ash and the Powers Caldera resemble modern Kilauea lavas in composition. Subsequent eruptions produced lavas that are more Mauna Loa-like in composition, or have compositions intermediate between those of modern Kilauea and Mauna Loa lavas. The greater thickness of these lavas in Kilauea Iki pit crater, in contrast with their restricted occurrence in Pauahi pit crater, is attributed to filling of the Powers Caldera and to the greater eruptive frequency in the summit region of the volcano compared with the upper east rift zone during this period. Only over the past 0.4 kyr have lavas resembling historical Kilauea lavas been erupted.

magma would retain its compositional identity in discrete magma batches and would subsequently be erupted. Much of the invading magma, however, would mix with residual pockets of Kilauea magma distributed throughout the plumbing system to produce magma with intermediate compositions. Table 1 and Fig. 2 show that such a mixing scenario is plausible. Subsequently, as Kilauea's magma supply recovered, eruptions of strictly Mauna Loa-like lavas would become increasingly rare and lavas of intermediate composition would predominate. Once initiated, such a magmatic connection might remain active for some time.

This model is at present based primarily on geochemical arguments. Seismic or geodetic observations show no evidence for currently active magma conduits between the two volcanoes, but there is tentative geophysical evidence for a fossil shallow magmatic connection. Aeromagnetic data display a roughly linear zone of high magnetic intensity from the vicinity of Kilauea caldera to the upper north-east rift zone of Mauna Loa, and have been interpreted as indicating an interconnection between the two volcanoes[30,31]. Furthermore, a recent seismic tomography study by Ho-Liu and Johnson (personal communication) identifies a sub-horizontal zone of high attentuation between the two volcanoes at a depth of 4–10 km that is roughly coincident with the aeromagnetic anomaly. As it is not presently active seismically, it clearly does not reflect a current magma conduit, but it could be the 'fossil' relict of the magma conduit required by our model.

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Fitness costs of gestation and lactation in wild mammals

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Like a number of plants[1,2], some mammals commonly produce more progeny than they can afford to rear, terminating investment in some or even all of their offspring once the resources available for breeding are known[3–5]. Adaptive interpretations of juvenile wastage rely on the argument that the costs of gestation are small compared to those of feeding offspring. Though energetic evidence supports this conclusion[6], it is unsafe to assume that the relative costs of gestation and lactation to the mother's survival and future reproductive success follow the same pattern because lactation commonly coincides with the period of maximum food availability. Controlling for individual variation, we show that in wild red deer (*Cervus elaphus* L) any costs of gestation to the mother's subsequent survival and reproductive success are slight compared to those of lactation.

Reproductive histories of 283 individually recognizable hinds were collected in a food-limited population on the Isle of Rhum, Inner Hebrides between 1971 and 1987 (ref. 7). In each year of

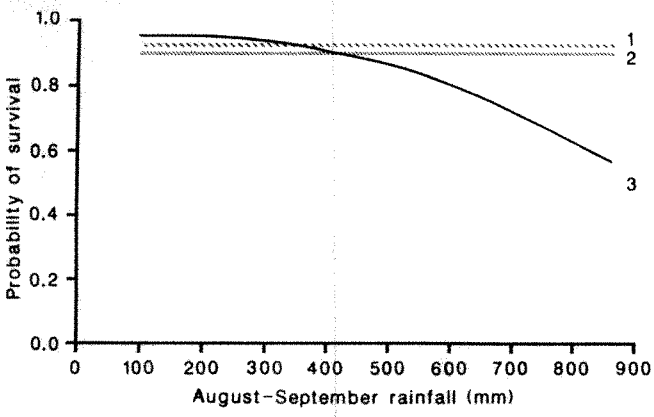


Fig. 1 Logistic curves illustrating the probability of a hind surviving a winter following a year in which she was (1) barren, (2) had produced a calf but lost it shortly after birth, and (3) reared a calf until weaning, are plotted against August-September rainfall (mm), which depresses survival in this population ($\chi^2 = 23.3$, $P < 0.001$) (ref. 9). These curves are standardized for hind age and values shown are for 12-year-olds (quadratic in age: $\chi^2 = 78.1$, d.f. = 2, $P < 0.001$). N (hind-years) = 1,479. Coefficients in the exponential equation (see text) are: (1) 3.107 − 0.000907 (August-September rainfall); (2) 2.444 − 0.000177 (August-September rainfall); (3) 3.563 − 0.003427 (August-September rainfall).

their lives, hinds were allocated to four categories. The first category consisted of those hinds that produced no calf (barren). Previous evidence suggests that when a hind was barren she had usually failed to conceive[7] and consequently did not suffer the costs of either gestation or lactation. The second category consisted of hinds that lost their calf within three months of its birth. In practice, 90% of the 156 such calves died within their first two weeks of life. In these years, mothers consequently avoided most of the energetic cost of lactation. The third category consisted of hinds whose calf had died between 4 and 12 months of age. 80% of the 204 calves allocated to this category died between February and May aged 9–12 months. Finally, the fourth category consisted of hinds that had successfully raised their calf to at least 12 months of age. We investigated relationships between the period for which the calf survived and four components of the mother's fitness: her survival during the winter following the calf's birth; her probability of producing a calf in the year after the calf's birth (fecundity); and the birth date and weight of her subsequent calf, both of which are known to affect calf survival[8]. In this population, high rainfall in August and September depresses the growth of grass and reduces food availability at the onset of winter, with the result that both fecundity and over-winter survival fall after wet autumns[9]. Our analysis consequently allowed for annual variation in rainfall in late summer.

Differences in fitness components between barren hinds and those whose calves died soon after birth should reflect the costs of gestation, whereas differences between mothers whose calves died soon after birth and hinds whose calves survived for six months or more should reflect the costs of lactation. Estimates of the costs of reproduction based on comparisons of this kind can underestimate the actual costs of breeding because, despite their greater energetic expenditure, mothers of superior phenotypic quality sometimes show higher survival rates or superior reproductive performance when compared to inferior phenotypes that did not breed in the previous year[10,11]. To minimize this problem, we compared the subsequent reproductive performance of the same hinds following years when they had been barren, had produced calves but lost them soon after birth or had reared calves to over six months.

In these analyses we included a factor to remove the effects of individual differences between mothers and restricted our sample to 145 females born between 1967 and 1978 to ensure that we included reasonable samples of breeding attempts for each individual. Generalized linear models[12] were used to estimate the probability of the mother's survival or fecundity in relation to her reproductive status in the previous year[13]. The dependent variables were binary (survive = 1, die = 0; fecund = 1, not fecund = 0) and must be related to any explanatory variable (such as summer rainfall) in a nonlinear way. A logistic model[14] was used to relate a particular transformation of the probability of survival or breeding for each individual (= the logarithm of the odds against dying or failing to breed) to a linear combination of explanatory variables. Logistic regressions were of the form:

$$P(Y_i =) = \frac{\exp(A + B_1x_{i1} + B_2x_{i2} + B_3x_{i3} + B_4x_{i4} + B_5x_{i5})}{1 + \exp(A + B_1x_{i1} + B_2x_{i2} + B_3x_{i3} + B_4x_{i4} + B_5x_{i5})}$$

where A, B_1, $B_2, \ldots, B_5$ = constants, x_{i1} = August-September rainfall (mm), x_{i2} = previous reproductive status, x_{i3} = reproductive status by rainfall interaction, x_{i4} = age (years), x_{i5} = age^2 and $i = 1, 2, 3, \ldots, N$ hind-years. Offspring birth weights and log-transformed calving dates were examined using an analysis of variance.

The fitness costs of gestation were evidently slight compared to those of lactation. In all years, the survival of barren females did not differ from those whose calves died during their first summer ($\chi^2 = 0.403$, NS). Except in years when late summer rainfall was unusually low and food availability in autumn was unusually high, both these categories of hinds were more likely to survive than mothers whose calves survived until weaning ($\chi^2 = 17.002$, $P < 0.001$, see Fig. 1; reproductive status by rainfall interaction: $\chi^2 = 6.046$, $P < 0.05$).

The subsequent fecundity of hinds did not differ between years when they had been barren and years when they had produced calves but lost them shortly after birth ($\chi^2 = 2.045$, NS). In contrast, their chances of calving were reduced after years in which they had suffered the costs of lactation (barren and early calf loss versus calf reared to >6 months, $x^2 = 292.21$, $P < 0.001$, see Fig. 2) and this difference increased with rainfall

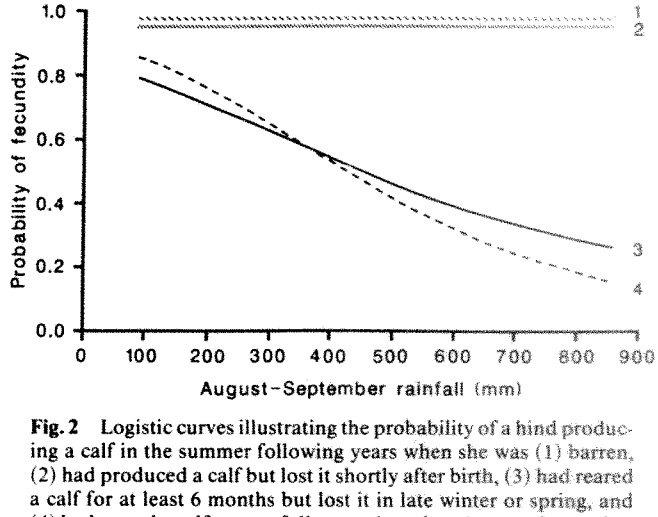


Fig. 2 Logistic curves illustrating the probability of a hind producing a calf in the summer following years when she was (1) barren, (2) had produced a calf but lost it shortly after birth, (3) had reared a calf for at least 6 months but lost it in late winter or spring, and (4) had reared a calf successfully, are plotted on August-September rainfall, which affects fecundity in this population ($\chi^2 = 29.1$, $P < 0.001$) (ref. 9). These curves are standardized for three variables that affect fecundity in this population: winter temperature ($\chi^2 = 12.2$, $P < 0.001$), values shown are for mean December-February daily temperature (4.5 °C); hind age (quadratic in age, $\chi^2 = 32.0$, d.f. = 2, $P < 0.001$), values shown are for 12-year-old hinds; and individual identity ($\chi^2 = 298.3$, d.f. = 134, $P < 0.001$), set here to the median individual. Coefficients in the exponential equation (see text) are (1) 3.728 − 0.000285 (August-September rainfall); (2) 3.319 − 0.000055 (August-September rainfall); (3) 1.637 − 0.003205 (August-September rainfall); (4) 2.160 − 0.004745 (August-September rainfall).

Table 1 Mean calving dates and offspring birth weights of red deer hinds

	Subsequent calving date		Birth weight of next calf (kg)	
Barren	5 June	NS	6.614	*
Calf produced but dies soon after birth	3 June		7.029	
		***		***
Lactating:				
Calf reared for at least 6 months but died between 6 and 12 months	14 June	NS	6.300	NS
Calf survives to 1 year old	14 June		6.466	

Mean calving dates and offspring birth weights of hinds following years in which they had been barren, produced a calf but lost it shortly after birth, reared a calf for at least 6 months but lost it between 6 and 12 months, and reared a calf which survived to one year. Means were standardized to the average individual and average year (1979 for calving date and 1978 for birth weight). In the case of offspring birth weight, means were adjusted for differences in calf sex ratio and the table shows values for males, which are born 0.38 kg heavier than females. NS, not significant; *, $P < 0.05$; ***$P < 0.001$.

in late summer (reproductive status by rainfall interaction, $\chi^2 = 13.1$, $P = 0.001$). Similarly, the subsequent calving date of hinds did not differ between years when they had been barren and years when they had produced calves but lost them shortly after birth ($t = 1.14$, NS), whereas on average they calved and, presumably, conceived ten days later when their previous calf survived to weaning ($t_{436} = 7.61$, $P < 0.001$; see Table 1). This change probably represented an appreciable cost, for a calf's chances of surviving the first year of life declined by 1% per day[15].

Finally, hinds produced heavier calves after years when they had been barren or lost their calves shortly after birth than following years when they had reared calves into the winter ($F_{2,286} = 5.517$, $P < 0.001$; see Table 1). However, hinds produced heavier calves following years when they had produced but lost calves than following those when they had been barren ($t_{102} = 2.04$, $P < 0.05$), presumably because failure to breed was associated with poor body condition and this had an effect on the hind's reproductive performance the following season.

Our results thus confirm that in food-limited populations the fitness costs of gestation are slight compared to those of lactation. It may often be to the advantage of females to produce more offspring than they can usually rear and subsequently to reduce the number of offspring to a level that they can support. This may explain why, in many species, juvenile survival declines more rapidly with resource shortage or with rising population density than fecundity[16].

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The potential role of pathogens in biological control

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It is now well established that pathogens such as viruses, fungi, bacteria and protozoans can have profound effects on the dynamics of their invertebrate host populations[1]. Theoretical[1,2] models of invertebrate host–pathogen interactions which assume uniform structure of the pathogen population may reasonably explain the oscillatory behaviour observed in some systems[3], but do not adequately describe the existence of more constant populations found in other host–pathogen interactions[4–7]. An examination of the literature relating to these relatively stable systems suggests that the common thread is the eventual transmission of some of the more protected, longer-lived stages of the pathogen occurring in reservoirs, such as the soil[4,5], host cadavers on trees[8], or the live host itself[7]. In this letter, I propose a new theoretical model which incorporates this population structure and accounts for the range of dynamics observed in natural systems. In particular, I show that host populations may be regulated to low and relatively constant densities if sufficient numbers of pathogens are translocated from pathogen reservoirs to habitats where transmission can occur. An understanding of pathogen reservoirs may be of value in the design of biological control programmes and may greatly increase the effectiveness of pathogens as biological control agents.

Models of invertebrate host–pathogen interactions often assume that the transmissibility and lifespan of the pathogen does not differ between pathogen individuals, or stages[1,2]. In nature, however, pathogen populations are far from uniform, because heterogeneities can exist in their spatial and genetic composition[9]. Models which assume homogeneity in pathogen populations have been used to explain the periodic cyclic behaviour of some forest Lepidoptera species[2,3], but the biological features of the pathogen responsible for these oscillatory dynamics (namely high pathogenicity and long-lived stages) are also characteristic of some systems in which the pathogen regulates its host to low and relatively constant densities[5,8,10].

The model presented here (Fig. 1) considers a more realistic structure of the pathogen population than earlier models by dividing the pathogen into two distinct subpopulations, one transmissible and short-lived, and the other non-transmissible and long-lived. For example, in the spruce sawfly, *Gilpinia hercyniae*, the transmissible stages of its nuclear polyhedrosis virus reside on the surface of spruce needles where they are exposed to vagaries of the environment, and are thus highly vulnerable to removal or to mortality[8,11]. Alternatively, stages of the virus can reside in more protected and remote habitats, such as under the bark of the trees, in the upper layers of the soil or in the cadavers of virus-killed hosts[8]. Stages in these 'reservoirs' can only cause infections if they are somehow translocated to the surface of the spruce needles by abiotic and biotic agents such as wind, rain, birds or the live host itself[8,12]. Although an obvious simplification of the real system, this scenario captures the essential dichotomy found in many invertebrate herbivore–pathogen interactions[5,8,13–17].

For the purposes of this study the invertebrate host is assumed to have continuous and overlapping generations, but the underlying results of the model also hold true for host taxa with discrete and non-overlapping generations. In addition, for the clarity of this presentation, the following assumptions are made[1]: (1) the risk of infection to a given host increases linearly with the density of the transmissible pathogen; (2) the production of pathogen stages in the host individual is directly proportional to the average lifespan of the infected host; (3) the infection is invariably lethal; (4) secondary infections are rare or do not

occur at all; and (5) the direct transmission of protected stages to susceptible hosts does not occur. Relaxing these assumptions does not change the basic conclusions of this study.

The dynamics of the host–pathogen interaction are described in terms of four differential equations which capture, in a general way, population processes which are similar to those described above in the invertebrate herbivore-pathogen scenario. These equations are for the density of susceptible hosts (X), infected hosts (Y), transmissible pathogen stages (W) and non-transmissible stages (Q) (Fig. 1): $dX/dt = (a_0 - b)X + a_1 Y - \beta XW$; $dY/dt = \beta XW - (\alpha + b - a_2)Y$; $dW/dt = \theta_1 Y - (\mu + \lambda)W + \nu Q - \phi\beta XW$; and $dQ/dt = \theta_2 Y - (\rho + \nu)Q + \lambda W$. Here a_0 and b are the per capita birth and death rates of susceptible hosts. Infected hosts give birth to either healthy or infected offspring at per capita rates of a_1 and a_2, respectively. The transmission parameter is β, and when infection takes place an average of ϕ stages are removed from the transmissible subpopulation of the pathogen. Once infected, the host can succumb either to natural causes of mortality (b) or to disease-related death (α). As the number of pathogen stages produced per infected host is assumed to be directly related to the average lifespan expected of the infected host[1,2] (that is, $1/(\alpha + b)$), the release of infective stages into the transmissible subpopulation and the reservoir can be represented by the steady states of $\theta_1 Y$ and $\theta_2 Y$ respectively. The transmissible stages die at a per capita rate μ or are translocated at a constant per capita rate of λ to the reservoir.

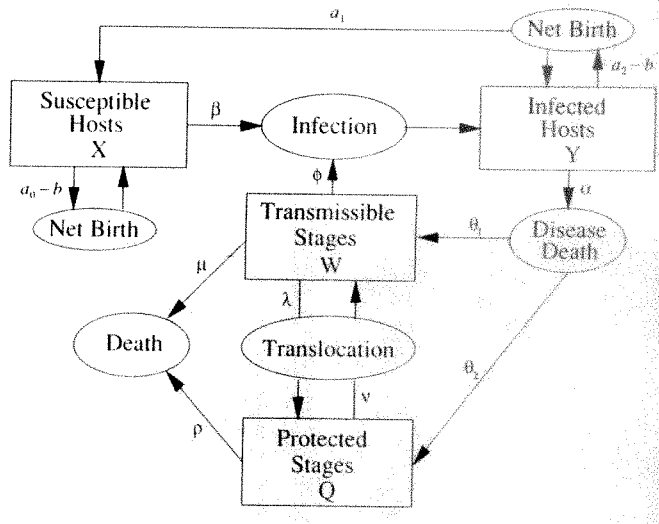


Fig. 1 Diagrammatic flow chart of transitions and rates in the interactions of susceptible hosts (X), infected hosts (Y), exposed, transmissible stages of the pathogen (W), and protected, non-transmissible stages (Q).

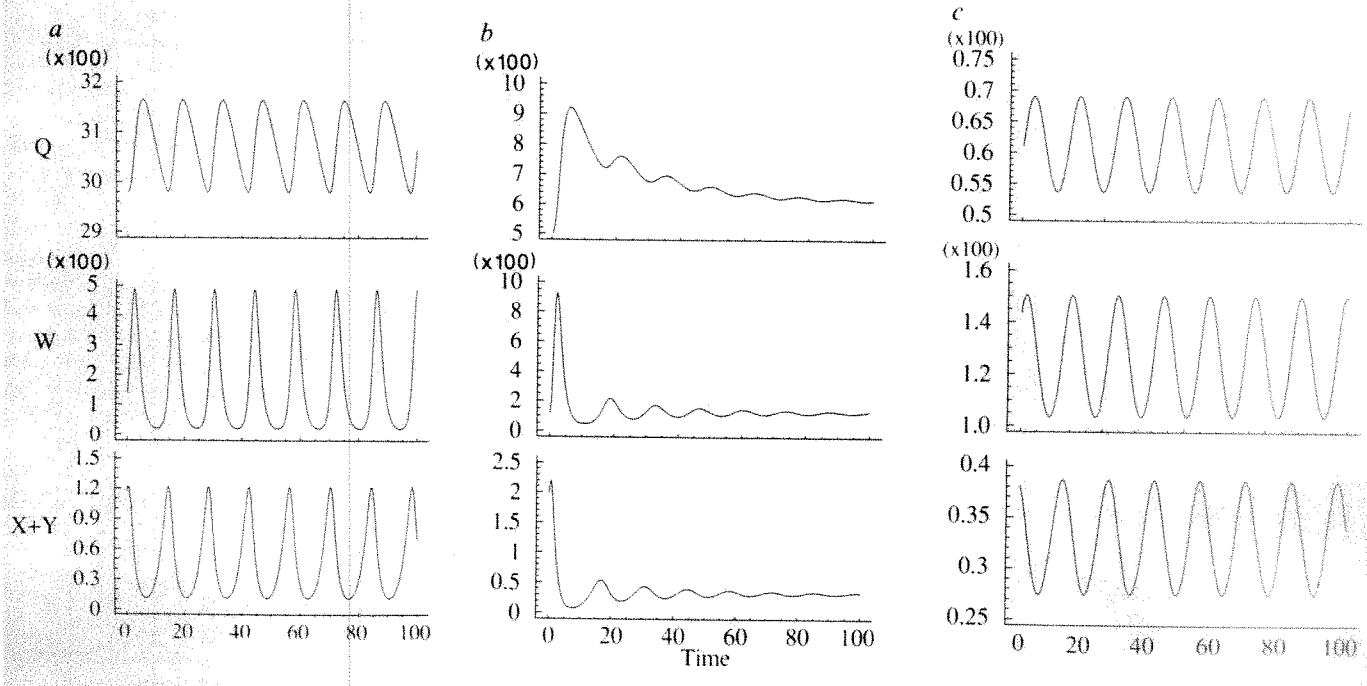


Fig. 2 Examples of population behaviour of total hosts ($X + Y$), transmissible pathogen stages (W) and protected stages (Q) for three different rates of flow of pathogen stages from the protected to the transmissible pathogen subpopulations. *a*, Low rates of flow ($\nu = 0$) resulting in oscillatory populations; *b*, intermediate rates of flow ($\nu = 0.04$) resulting in constant populations; *c*, high rates of flow ($\nu = 0.4$) giving rise to oscillatory populations. The effect of the pathogen reservoir on the host's dynamics can be intuitively understood as follows. First imagine a situation in which pathogen stages are not translocated from the reservoir to the transmissible subpopulation (corresponding to $\nu = 0$). The dynamics of the interaction are similar to those found by Anderson and May[1,2]: persistent oscillations with a period determined to some extent by the rate of liberation of pathogen stages into the transmissible subpopulation (θ_1) and the average lifespan of transmissible stages ($1/(\mu + \lambda)$). Add to this an intermediate flow of stages from the reservoir to the transmissible subpopulation. Now, at peak populations of the transmissible pathogen, stages are being translocated to and stored in the more protected habitats. At times when the transmissible stages are sparse, the release effects of the reservoir result in a net flow of stages to the transmissible subpopulation. Thus, the storage and release capacities of the reservoir tend to 'buffer' the transmissible subpopulation of the pathogen through time, resulting in constant populations of both host and pathogen at equilibrium. If the flow from the protected to the transmissible subpopulation is too strong, then the two pathogen subpopulations effectively become a single entity and the host and pathogen interact in much the same way as they did in the first scenario (that is, oscillatory populations). The important difference is that the greater input of pathogen stages into the transmissible subpopulation has the effect of depressing the host population to lower levels than in the first case (compare Fig. 2*a* and *c*). Parameters common to all three simulations are $a_0 - b = 0.5$; $a_1 = 0.2$; $\beta = 0.005$; $\phi = 1$; $\alpha + b - a_2 = 1$; $\theta_1 = 10^3$; $\theta_2 = 0$; $\lambda = 0.2$; $\mu = 1$; and $\rho = 0.001$.

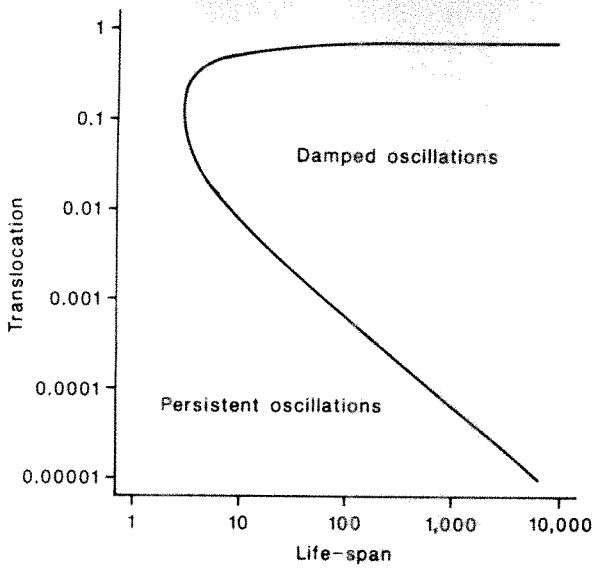


Fig. 3 Population dynamics as determined by the average lifespan of pathogen stages in the reservoir $1/\rho$, and the rate of translocation from the reservoir to transmissible subpopulation ν. Two types of population behaviours occur: oscillatory (unshaded) and constant (shaded) trajectories. Although the boundaries of oscillatory and constant populations will depend to some extent on the values of the other model parameters, there is a simple linear relationship between the logarithms of $1/\rho$ and ν through most of the parameter space. Estimation of these two crucial parameters[25,26] can be made from measurements of the rate of translocation of the pathogen from the reservoir to the transmissible subpopulation (ν) and the average duration of the activity of the pathogen in the reservoir ($1/\rho$ or $1/(\rho+\nu)$). Anderson[27] has found that if the extent of artificial introduction of a pathogen into the host population is sufficient, then it can result in the dampening of cyclic populations. This is very similar to the effect of adequate translocation from the pathogen reservoir to the transmissible subpopulation. Values for other parameters are as given for Fig. 2, except that $\lambda = 1$.

In a similar way, stages residing in the reservoir die (ρ) or are translocated at a constant per capita rate ν to the transmissible subpopulation.

The properties of the model are straightforward. There is a single positive equilibrium only if the pathogen is sufficiently virulent (that is, $\alpha > a_1 + a_2 - b$) and if the number of stages removed from the transmissible subpopulation when infection occurs is sufficiently small. As other forms of population regulation are not included in the model, in the absence of the disease the host population grows unregulated at an exponential rate, $a_0 - b$.

As illustrated in Figs 2 and 3, the model exhibits one of two distinct types of behaviour at equilibrium: (1) stable oscillations or (2) constant population densities. The basic features of the model that determine which of these prevail are the storage and release of pathogen stages in the reservoir (governed primarily by constants λ, θ_2, $1/\rho$ and ν). Very low values of these potentials give rise to stable oscillations (Figs 2*a* and 3). As they are increased to intermediate levels, the result is constant host and pathogen populations (Figs 2*b* and 3). Finally, increasing the release potential of the reservoir (governed by the constant ν) even further produces, once again, cyclic populations (Figs 2*c* and 3). But further increases in the storage of stages (governed by the constant $1/\rho$) continues to enlarge the parameter space for constant host populations (Fig. 3). Changes in other parameters of the model may affect the boundaries of the stable and unstable equilibrium, but do not alter the central results.

There are number of real examples which support the results presented here[4-7,13-15]. The most striking of these comes from the interaction between Hepialid moths of the genus *Wiseana* and their nuclear polyhedrosis virus[4,10,18-21]. The larvae of these moths are pests of grasses in pastures in New Zealand. In long-term pasture leys, larvae that die from the disease accumulate in the upper layers of the soil to form a reservoir. Translocation of the virus from this reservoir to the larval food source (grass swards) has been attributed to the grazing of animal stock. The populations of the pest in these pastures are low and fairly constant from year to year. But management practices which disturb the pathogen reservoir (such as ploughing the pastures) and/or reduce the translocation of the pathogen from the reservoir to the grass swards (no grazing, for example) result in outbreaks of the pest, with highly variable populations from year to year. If both of these disruptive practices are halted, then the pathogen reservoir slowly builds, accompanied by the depression of the pest population. Thus, the creation of a distinct reservoir along with specific mechanisms for mobilization of the pathogen into the host population seems to mediate the differences in dynamical behaviour, which is in agreement with the model proposed here. Other real systems where pathogen reservoirs have an apparently similar role in the biological control of arthropod pests are: the Japanese beetle, *Popillia japonica*, and its bacterium, *Bacillus popilliae*[5]; the spruce sawfly, *Gilpinia hercyniae*, and its nuclear polyhedrosis virus[6]; the palm rhinoceros beetle, *Oryctes rhinoceros*, and its rhabdovirus[7]; the velvetbean caterpillar, *Anticarsia gemmatalis*, and its nuclear polyhedrosis virus[13]; the grasshopper, *Zonocerus variegatus*, and its fungus, *Entomophthora grylli*[14]; and the cabbage looper, *Trichoplusia ni*, and its nuclear polyhedrosis virus[15]. It should be stressed that, in these examples, the details of the mathematical model (for example, the amplitude and period of the cycles) are very difficult to corroborate because of the requirements of long runs of population data and accurate estimates of the parameters of the model. Rather, the crucial prediction made by the model is a strategic one: increasing the levels of release of pathogen stages from established reservoirs to the habitat of the host should lead to lower and more constant populations.

The model presented here constitutes a realistic framework for predicting the consequences of potential strategies in biological control programmes. Equally important, the model serves to clearly identify the biological factors that should be measured and manipulated, whether for experimentation or the actual practice of biological control. Measures[22] to increase the efficacy of indigenous pathogens of arthropod pests should focus on the identification and manipulation of pathogen reservoirs and the processes that move pathogens between non-transmissible and transmissible subpopulations. In particular, the conservation of potential vectors of the pathogen should be paramount. For the introduction of exotic pathogens as 'classical' biological control agents, the model suggests that the conditions for determining their likelihood of success are (1) the long lifespan of pathogen stages residing in reservoirs and (2) the propensity of these stages to be translocated to the habitat of the host for possible transmission. Thus, the screening of promising agents and systems with pathogen reservoirs in mind is a sensible practice. In the past, microbial biological control programmes[23,24] have focused on measures which yield short-term control of arthropod pests. These results indicate that long-term control is possible and may not entail much cost or effort.

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Maturation and connectivity of the visual cortex in monkey is altered by prenatal removal of retinal input

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In several species, the peripheral input from the eyes partly determines the pattern of interconnections between the visual areas of the two cerebral hemispheres through the fibre tract termed the corpus callosum[1-9]. In the macaque monkey, the neurons projecting through the callosum are largely restricted to area 18 throughout ontogeny, whereas area 17 is characterized by few or no callosal projections[10-12]. Here, we show that suppressing the peripheral input by prenatal removal of the eyes leads to a marked reduction in the extent of area 17, resulting in a large shift in the position of the histologically identifiable boundary between the two areas. Even so, the boundary continues to separate an area rich with callosal connections (area 18) from one poor in such projections (area 17), indicating there is no effect on the callosal connectivity of area 17. In contrast, in area 18, eye removal results in many more neurons with callosal projections than in normal animals. The results suggest that the factors that determine the parcellation of cortical areas also specify their connectivity.

Two fetal macaque monkeys (*Macaca cynomolgus*) had both eyes removed at 77±2 and 112±2 days after conception (days E77 and E112), and the fetuses were then replaced in the uterus. At about the normal time of birth the callosal connectivity of areas 17 and 18 was determined using horseradish peroxidase histochemistry.

The pattern of layers in area 17 seemed remarkably normal in both experimental animals. The border between areas 17 and 18, clearly defined in Nissl-stained sections, was as distinctive as in the neonatal control (Fig. 1 and ref. 13). The 17/18 border could also be detected in sections reacted for cytochrome oxidase activity (Fig. 1). Comparison of adjacent sections stained by the two methods reveals a double band of higher cytochrome oxidase activity in layer 4C that stops abruptly at the 17/18 border, as in the control animal[14]. Despite the weaker staining for cytochrome oxidase activity in the experimental animal its distribution within area 17 was, on the whole, similar[14].

In the monkey operated on day E77, the gyral pattern of the occipital lobe was unusual and the surface area of area 17 was considerably reduced (Fig. 2, see also ref. 13), particularly laterally where, in the intact animal, cortex subserving foveal and parafoveal vision is located. Later eye removal (on day E112) did not cause a noticeable reduction in the surface area of area 17 or a disturbance in the gyral pattern[13].

In both animals after eye removal, the disposition of callosally projecting neurons with respect to the border between areas 17

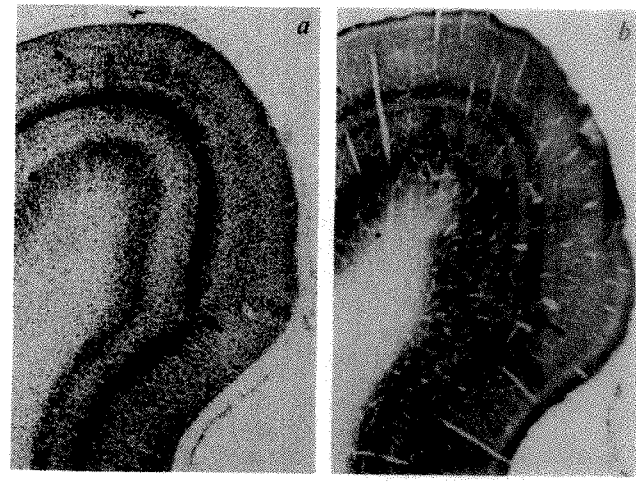


Fig. 1 Characteristics of the 17/18 border after early eye removal (E77). *a*, Section stained with cresyl violet *b*, Adjacent section reacted to show cytochrome oxidase activity. The arrows indicate the 17/18 border. Although the level of cytochrome oxidase activity is lower than in normal neonates the overall distribution in area 17 and the change in layer 4 at the 17/18 border is similar.
Methods. Timed pregnant monkeys were prepared for surgery under ketamine (i.m.) followed by Alfatesin (i.v.) anaesthesia. After intubation, anaesthesia was continued with halothane in a N_2O/O_2 mixture (70:30). Expired CO_2 and heart rate were monitored. The body temperature was maintained using a thermostatically controlled heating blanket. A midline abdominal incision was made and uterotomy performed. After exposure of the fetal head and bilateral eye removal, the fetus was replaced in the uterus and incisions closed using routine procedures. The mother was returned to her cage and medicated for the first two days with analgesic (Visceralgine, i.m.). A muscular relaxant (Duvadilan, i.m.) was given twice daily until the fetus was delivered by caesarian section 1-2 weeks before term. The infant monkey was bottle fed until the axonal tracing experiments on day E159 when removal was at E77, and E164 when eye removal was at E112 (the gestation period is 165 days). Infant monkeys were anaesthetized with ketamine (i.m.) followed by Alfatesin (i.v.). Craniotomy was performed over the occipital lobe and 15-25 μl of 30-50% horseradish peroxidase (HRP) were injected using a Hamilton syringe equipped with a glass micropipette (60-120 μm tip diameter). Closely spaced injections were made over the occipital lobe so as to massively fill area 17 and the adjacent part of area 18. After 24 hours, the infant monkey was deeply anaesthetized and perfused through the heart with a mixture of 1.25% paraformaldehyde and 1.5% glutaraldehyde. Sections (60 μm thick) were cut parasagitally and one in three sections were processed for HRP histochemistry[18]. Intermediate sections were processed for cytochrome oxidase[19] and stained for Nissl substance (cresyl violet). The border of area 17 and 18 was clearly defined in Nissl and cytochrome oxidase-reacted sections and in the hemisphere contralateral to the injection of HRP, labelled neurons were counted and allocated to either area. Counts of labelled neurons were made in area 18 from the 17/18 border to the fundus of the lunate sulcus. Individual sections were traced out and the positions of labelled cells recorded using an *x-y* plotter electronically coupled to the microscope stage.

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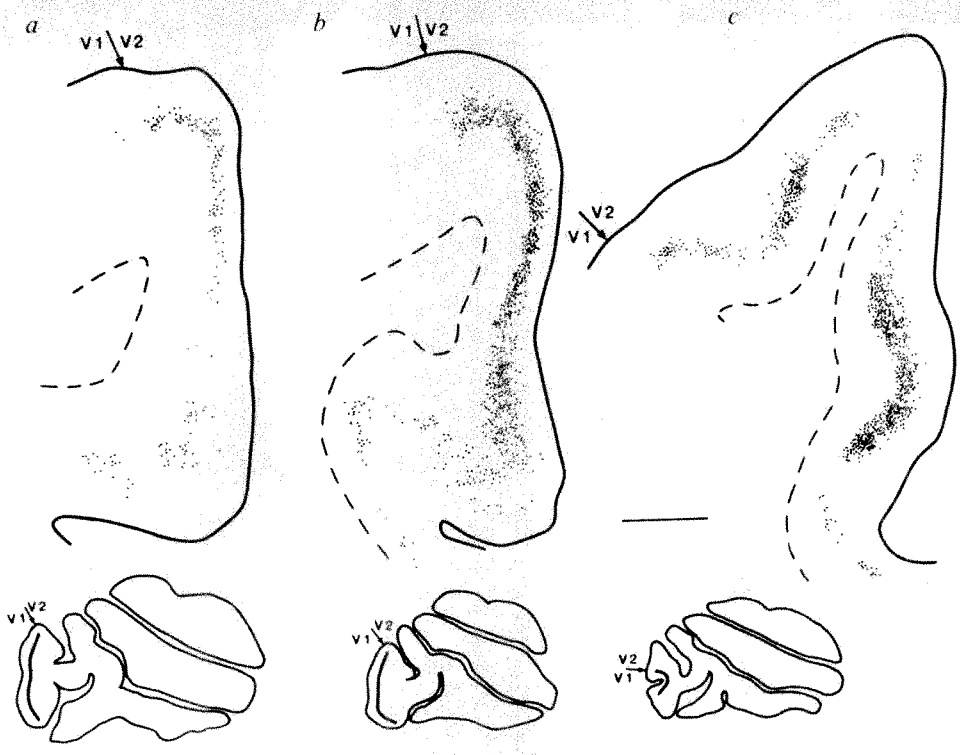


Fig. 2 Parasagittal sections of the area 17/18 border showing callosally projecting cells in the newborn monkey. *a*, Normal. *b*, Eye removal at E112. *c*, Eye removal at E77. Each dot represents a retrogradely labelled cell and arrows indicate the area 17/18 border. Inserts show low-power drawing at the level at which the high-power plots were made and where the continuous line in the operculum indicates the extent of area 17. Eye removal did not increase the numbers of callosally projecting neurons in area 17, but did increase the numbers of these cells in area 18. Whereas area 17 occupies most of the operculum of the normal animal and after eye removal at E112, after eye removal at E77, area 17 seems considerably shrunken. Scale bar, 1 mm.

and 18 was normal (Fig. 2): they were largely confined to the extrastriate cortex and stopped abruptly at the 17/18 border. In both animals, only one or two labelled neurons per section were found in area 17 where they were located, as in normal animals, within 2–3 mm of the border[11,15,16]. In contrast, in area 18, callosally projecting neurons were more densely packed and stretched further rostrally from the 17/18 border than in normal neonates (Fig. 2). Counts of callosally projecting neurons were made in the part of area 18 that extends from the 17/18 border to the fundus of the lunate sulcus, in the part of the cortex that represents the parafoveal region of the visual field. In the normal animal there were 50–140 labelled neurons per section[10,12], whereas in both experimental animals the number was 5–6 times higher (280–875) than in either normal neonates or adults (Fig. 3). Although the age at which the eyes were removed did not influence the numbers of callosally projecting neurons in area 18, it did affect their spatial distribution. The animal operated on day E112 showed a continuous distribution of callosally projecting neurons but in the animal operated on day E77 the distribution of callosally projecting neurons was discontinuous and patchy (Fig. 2).

The demonstration that eye removal alters the callosal connectivity of area 18 but not of area 17 should be considered in the light of the ontogeny of callosal connections in the monkey. Several studies have shown that area 17 is largely free of callosal connections throughout development (ref. 11; L. M. Chalupa and H. Killackey manuscript, in preparation; P. Rakic, personal communication). Eye removal, which stabilizes transient callosal connections in all other species examined[1–9], in monkey fails to reveal a transient callosal projection in area 17, emphasizing that the lack of callosal connections in area 17, unique to the monkey, is specified early in development. The normal maturation of the cortical connectivity of area 18 differs from that of area 17, as the distribution of callosally projecting neurons in area 18 changes during development[11]. Because eye removal both early and late in prenatal life leads to higher numbers of callosally projecting neurons in area 18, the elimination of the transient callosal projection, which normally occurs in the last month of gestation[11] must be regulated by epigenetic factors that depend at least partially on peripheral input.

The reduced size of area 17 after early eye removal could result from an increase in cell death. As the number of afferent fibres from the lateral geniculate nucleus in the thalamus is reduced after early eye removal (ref. 17; H. Kennedy, unpublished observation), the survival of neurons in area 17 may depend critically on the density of the input. It is also possible that the input from the thalamus contributes directly to determining cortical areas, so that the smaller number of afferents from the lateral geniculate nucleus after eye removal leads to reduction in the extent of cortex specified as area 17 and a consequent expansion of the adjacent area 18[13]. This raises the question of whether the cortex immediately adjacent to area 17 constitutes

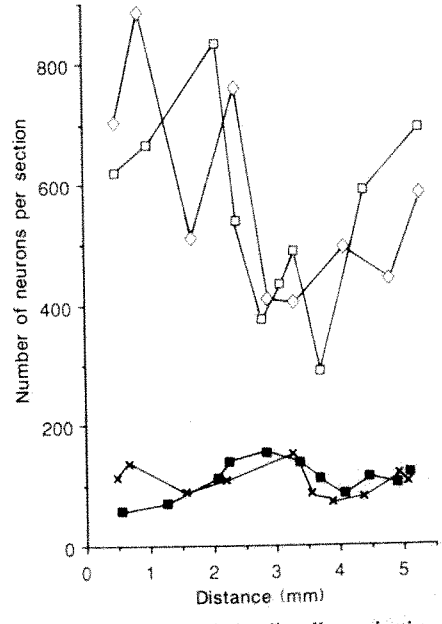


Fig. 3 Counts of HRP labelled callosally projecting neurons in area 18 of normal adult (■), neonatal control (×), after eye removal at E77 (□) and after eye removal at E112 (◇). In normal development, the numbers of callosally projecting neurons do not change after birth. Counts of callosally projecting neurons were considerably higher in the two animals whose eyes were removed. The age at which the eyes were removed had no influence on the numbers.

a hybrid area, that is, a region of cortex with characteristics of both areas 17 and 18[13]. As the absence of callosal connections from area 17 is determined early in development, it can be used to indicate whether tissue originates developmentally from area 17 or area 18. As callosally projecting neurons are found in area 18 immediately adjacent to area 17, the expanded area 18 seems to be a cortical region that was unspecified when the eyes were removed and, because of the subsequent lack of peripheral input, failed to mature into area 17.

These results indicate that retinal input has several, somewhat independent effects on cortical development. Early in development the factors that help to specify the limits of cortical visual areas also regulate the overall presence or absence of callosal connectivity. Once the boundaries of areas are established, the influence of peripheral input is limited to regulating the density of callosal projection neurons in extrastriate visual cortex.

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Effect of recombinant soluble CD4 in rhesus monkeys infected with simian immunodeficiency virus of macaques

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The CD4 molecule is a high-affinity cell-surface receptor for the human immunodeficiency virus (HIV-1)[1] and a soluble truncated form of CD4 produced by recombinant DNA technology is a potent inhibitor of HIV-1 replication and HIV-1-induced cell fusion *in vitro*[2–6]. Rhesus monkeys infected with the simian immunodeficiency virus of macaques (SIV_{MAC}), a virus closely related to HIV-1, develop an AIDS-like syndrome, and so provide an important model for the evaluation of potential AIDS therapies[7]. We have assessed the therapeutic effect of recombinant soluble CD4 in SIV_{MAC}-infected rhesus monkeys. Virus was readily isolated from peripheral blood lymphocytes and bone marrow cells of these animals before starting treatment with soluble CD4, but became difficult to isolate soon after treatment had begun. Moreover the diminished growth of both granulocyte-macrophage and erythrocyte progenitor colonies from the bone marrow of these monkeys rose to normal levels during treatment. These findings indicate that soluble CD4 could prove valuable in the treatment of AIDS.

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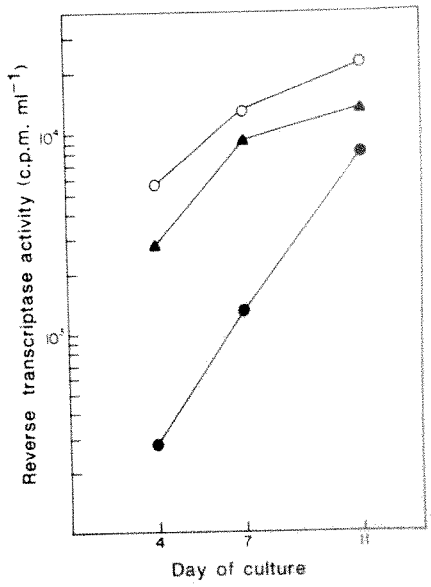


Fig. 1 Inhibition by rsCD4 of SIV_{MAC} infection of normal rhesus monkey PBLs *in vitro*. SIV_{MAC}-containing culture supernatant was incubated for 1 h at 37 °C with culture medium alone or with two concentrations of rsCD4 and then added to 2.5×10^6 cell aliquots of concanavalin A activated normal rhesus monkey PBLs in 1 ml, resulting in final concentrations of no rsCD4 (○), 12.5 μg ml^{-1} rsCD4 (▲), and 125 μg ml^{-1} rsCD4 (●). After a 2-h incubation at 37 °C, these cells were brought up to 5 ml in IL-2-supplemented medium. Following an overnight incubation, the cell populations were washed, resuspended in IL-2-supplemented medium at 5×10^5 cells per ml and maintained at 37 °C in a humidified 5% CO_2 atmosphere. Culture medium was collected every 3–4 days and assayed for reverse transcriptase activity[14].

Our growing understanding of HIV-1 and its interaction with the cells it infects is leading to rational strategies for AIDS therapy. The CD4 molecule acts as a high-affinity receptor for the outer viral envelope glycoprotein (gp120) of HIV-1[1]. Monoclonal anti-CD4 antibodies block the ability of HIV-1 to infect target cells *in vitro*[8,9], and the transfer and expression of the CD4 gene to previously $CD4^-$ human cells confers susceptibility to HIV-1 infection on those cells[10]. It has recently been shown that a recombinant soluble form of the CD4 molecule (rsCD4) is a potent inhibitor of both HIV-1 replication and virus-induced cell fusion *in vitro*[2–6]. These findings led to the proposal that rsCD4 may be useful in AIDS therapy.

Lentiviruses related in their nucleotide sequences to the human AIDS viruses HIV-1 and HIV-2 have recently been isolated from African and Asian non-human primates[11]. Some of these isolates, when inoculated into rhesus monkeys, induce a disease with striking similarities to human AIDS[7,12,13]. The SIV_{MAC}-induced disease in the rhesus monkey has already proved to be an important model for studying the pathogenesis of AIDS and for assessing strategies for vaccination and therapeutic interventions in this disease[14,15]. The human and macaque CD4 molecules are strikingly similar both in their monoclonal anti-CD4 epitope mapping and in their amino-acid sequences that define the minimal region for binding the outer envelope glycoprotein of the AIDS virus (R.A.F. *et al.*, unpublished data). In agreement with these observations, we find that a recombinant soluble human CD4, a 375-amino acid protein comprising the entire extracellular portion of the molecule[2], blocks rhesus monkey peripheral blood lymphocyte (PBL) infection by SIV_{MAC} *in vitro* (Fig. 1). Although human rsCD4 may

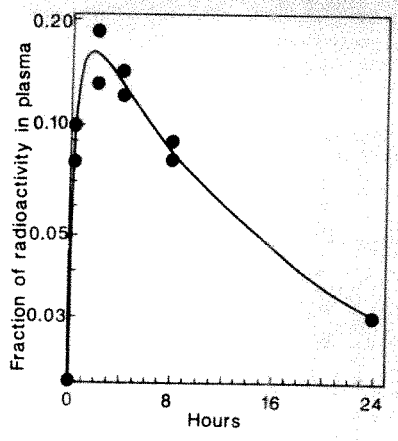


Fig. 2 Plasma clearance of total radioactivity from 2 rhesus monkeys following intramuscular administration of ^{125}I-labelled rsCD4. Radiolabelled rsCD4 (1.1×10^7 c.p.m.) was administered with 2 mg unlabelled rsCD4, i.m., into each of 2 normal 6.9 kg rhesus monkeys. Samples of plasma (1 ml) were regularly analysed for radioactivity. The fraction of the total initial radioactivity in the plasma was calculated assuming a plasma volume of 40 ml kg^{-1}.

be less efficient in blocking SIV_{MAC} infection of rhesus monkey PBLs than HIV-1 infection of human PBLs, the SIV/rhesus monkey model provides a reasonable system in which to assess the therapeutic potential of rsCD4 in AIDS.

Pharmacokinetic studies were performed to establish an appropriate route and frequency of administration of rsCD4 to the rhesus monkey. Animals weighed 5–7 kg. Two normal rhesus monkeys were given a single 2 mg intramuscular (i.m.) inoculation of rsCD4 with a trace quantity of ^{125}I-labelled rsCD4 admixed. As shown in Fig. 2, >10% of the calculated maximum possible fraction of radioactivity was found in plasma 15 min after a single dosage and persisted for 8 hours. Peak plasma levels of >1,400 ng ml^{-1} of rsCD4 were measurable in an enzyme-linked immunosorbent assay two hours after its administration. This method of delivery yielded an estimated rsCD4 serum half life of about 6 hours. Two uninfected rhesus monkeys were then given a 50-day course of rsCD4 treatment, each receiving 2 mg, i.m., daily, and did not experience any significant adverse reaction to the rsCD4. The distribution of lymphocyte subsets in their peripheral blood ($CD2^+$, $CD4^+$, $CD8^+$, IL-2-receptor$^+$, $B1^+$) and the function of those cells (proliferation following stimulation with xenogeneic cells and pokeweed mitogen) did not change during the course of treatment. Serum chemistry studies to assess liver and renal function on days 8, 15 and 44 of treatment and on days 8 and 18 after treatment were unchanged when compared with baseline values. The animals did experience a transient drop in the number of bone marrow granulocyte-macrophage progenitor (CFU-GM) colonies with an associated transient neutropenia, but this transient bone marrow suppression never became clinically significant.

Four SIV_{MAC}-infected rhesus monkeys were then treated with rsCD4 in the same way. Three of these virus-infected monkeys had nearly normal immune parameters at the start of treatment; the fourth had a circulating absolute $CD4^+$ lymphocyte count of less than 100 per mm^3 (normal rhesus monkeys have >1,000 $CD4^+$ PBLs per mm^3). None of the SIV_{MAC}-infected monkeys showed changes during treatment in the distribution of lymphocyte subsets in their peripheral blood, in PBL function or in serum chemistry assessment of liver and renal function. The SIV_{MAC}-infected animal (Mm167-84) that showed evidence of severe immunological impairment at the start of the study died on the third day after the end of treatment. There was evidence of a severe Coombs positive haemolytic anaemia. In the last 10 days before death, the animal's haemoglobin dropped from 7.7 to 3.7 g dl^{-1}. Lesions seen at necropsy included a large amount of clotted blood in the small intestine, severe myocardial fibrosis, and histiocytic infiltration of the splenic lymphoid follicles. These last two findings are not uncommon in rhesus monkeys chronically infected with SIV_{MAC}. The presence of blood in the proximal small bowel indicated that the animal could have died from an acute gastrointestinal haemorrhage, which was possibly secondary to stress induced by the acute haemolytic process. As significant haemolytic anaemias have been reported in AIDS[16], it is impossible to determine whether this death was a natural consequence of the SIV_{MAC} infection or was drug-induced.

The results of the virus-isolation studies in these four monkeys were striking (Fig. 3). Co-cultivation of PBLs from animals Mm156-85, Mm159-86 and Mm202-84 with H9 yielded SIV_{MAC} before treatment and on every isolation attempt up to about the end of week 2 of treatment. These virus isolation attempts thereafter were negative until one month after completion of treatment at the earliest. SIV_{MAC} replication in bone marrow cultures, a reflection of virus replication in macrophages rather than in T lymphocytes (data not shown) showed parallel

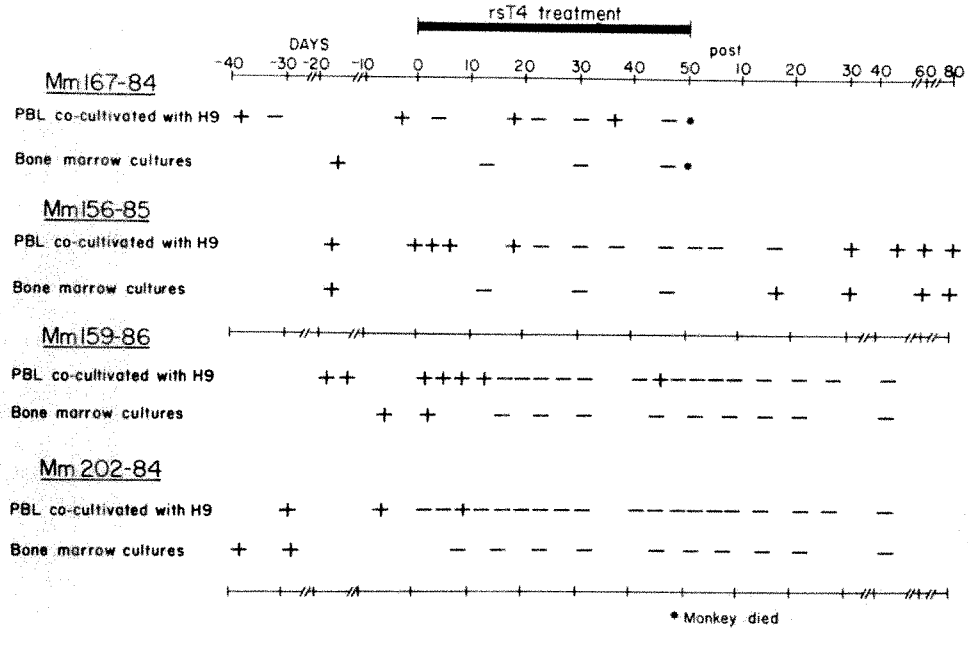


Fig. 3 SIV_{MAC} could not be isolated from PBLs and bone marrow of the virus-infected monkeys during treatment with rsCD4. PBLs of SIV_{MAC}-infected animals were isolated by Ficoll-diatrizoate density gradient ($\rho = 1.077$) centrifugation, activated with concanavalin A and co-cultured with the human T-cell line H9. Bone-marrow samples were obtained by posterior iliac crest aspiration from ketamine anesthetized monkeys and heparinized. Cells were isolated by density gradient centrifugation, washed and placed in culture in Iscove's modified Dulbecco's MEM supplemented with 12.5% FBS and 12.5% horse serum in 2 or 4 chamber Lab-Tek tissue culture slides (Miles Scientific) at a cell concentration of 1×10^6 per ml. After 7 days of culture, non-adherent cells were removed. In both culture systems, medium was changed regularly and supernatants were assayed for reverse transcriptase activity as previously described[14]. Supernatants with reverse transcriptase activity at least 5 times above background were scored as positive for infection.

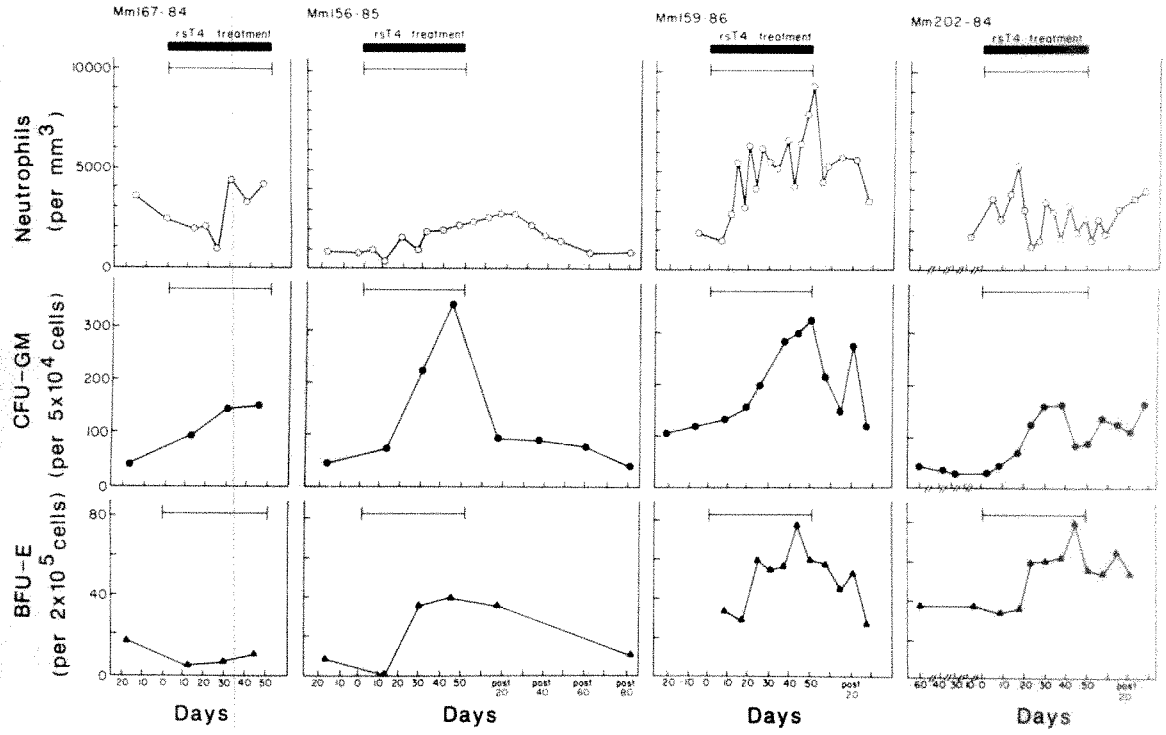


Fig. 4 SIV_{MAC}-infected rhesus monkeys demonstrate an increase in bone marrow granulocyte-macrophage and erythrocyte progenitor cell growth following rsCD4 treatment. A two-layer culture was established for the quantitation of CFU-GM colonies from bone marrow cells isolated as described in Fig. 3. The underlayer of 0.5% Noble agar contained 60 ng ml^{-1} recombinant human granulocyte-macrophage colony stimulating factor (kindly provided by Genetics Institute, MA). The overlayer contained 10^5 bone marrow cells in Iscove's modified DMEM supplemented with 12.5% FBS and 12.5% horse serum in 0.3% Noble agar. It was layered on to the underlayer in 35 × 10 mm tissue-culture dishes and the cultures were maintained at 37 °C in a 5% CO_2-humidified atmosphere. BFU-E colonies were assessed in cultures of bone marrow cells maintained in 0.9% (w/v) methylcellulose in Iscove's modified DMEM supplemented with 30% FBS, 0.9% deionized bovine serum albumin and 5×10^{-5} M 2-mercaptoethanol, containing 60 ng ml^{-1} granulocyte-macrophage colony stimulating factor, 1 unit ml^{-1} sheep erythropoietin (step III, Connaught Lab., Ontario) and 5% phytohaemagglutinin-stimulated rhesus monkey lymphocyte conditioned medium. CFU-GM and BFU-E colonies of >50 cells were then counted under an inverted microscope 12–14 days after establishing the cultures. Total neutrophil counts in the peripheral blood were determined from complete blood counts and differentials from EDTA-anticoagulated blood samples.

changes. Virus was readily isolated from bone marrow before treating these 3 monkeys, but attempts to isolate virus during treatment were negative. Virus isolations from bone marrow reverted to positive on day 18 after completion of treatment in Mm156-85, but remained negative until 43 days after treatment in Mm159-86 and Mm202-84. Virus was isolated only episodically from PBLs of the SIV_{MAC}-infected rsCD4-treated animal Mm167-84 following co-cultivation with H9 cells both before and during treatment (Mm167-84 was the animal that was immunologically compromised at the outset of the study and died 3 days after completion of treatment). But although isolation of virus from the bone marrow of Mm167-84 was positive before treatment, 3 attempts to isolate virus from the bone marrow during treatment were unsuccessful.

These negative virus isolations alone were not unequivocal evidence for a therapeutic effect of rsCD4; the rsCD4 could simply have interfered with the *in vitro* isolation of SIV_{MAC} without affecting virus replication or the sequelae of these infections in the monkeys. But the fact that virus isolations remained negative in three animals for longer than a month after treatment argues against this possibility. Moreover, functional abnormalities reflecting SIV_{MAC}-induced disease also improved in these animals. The *in vitro* growth of both CFU-GM and erythrocyte progenitor (BFU-E) colonies from the bone marrow of these monkeys was depressed at the start of rsCD4 treatment. This is a common finding in SIV_{MAC}-infected monkeys (data not shown) and in HIV-1-infected humans[17]. (CFU-GM colony counts in a series of studies of 10 normal animals were 222 ± 16 per 5×10^4 cultured bone marrow cells; BFU-E counts were 76 ± 4 per 2×10^5 cultured bone marrow cells.) The number of colony-forming cells from the bone marrow of these animals increased during rsCD4 treatment (Fig. 4). A bone marrow cell CFU-GM colony count of 48 per 5×10^4 cells before treatment of Mm167-84 rose during treatment to 146 per 5×10^4 cells with no significant change in the number of BFU-E colonies. The CFU-GM colony count derived from bone marrow of Mm156-85 rose from 44 per 5×10^4 cells before the start of therapy to 350 per 5×10^4 cells, with a coincident rise in BFU-E colonies from 8 to 40 per 2×10^5 cells. Bone marrow from Mm159-86 showed a rise in CFU-GM colonies from 106 to 329 per 5×10^4 cells and in BFU-E colonies from 34 to 78 per 2×10^5 cells. Bone marrow from Mm202-84 gave an increase in CFU-GM colonies from 46 to 163 per 5×10^4 cells and in BFU-E colonies from 38 to 80 per 2×10^5 cultured cells. A decline in bone marrow CFU-GM and BFU-E colony counts to baseline values occurred in the three surviving animals after stopping treatment. A transient neutropenia occurred in two of the four monkeys during the first two weeks of treatment. But there was an eventual rise in total peripheral blood neutrophil count in all four animals that accompanied the increased colony-forming cell count from the bone marrow.

The rsCD4 treatment of the two normal rhesus monkeys unexpectedly had an anti-viral effect. More than 90% of bone marrow cultures established from uninfected monkeys can be reproducibly infected with exogenously introduced SIV_{MAC} (data not shown). But although bone marrow from both the rsCD4-treated normal animals was susceptible *in vitro* to infection with SIV_{MAC} introduced before and 4 weeks after treatment, 3 attempts to infect bone marrow cell cultures from the animals during the 50-day treatment period and a single attempt 18 days afterwards gave negative cultures.

Our inability to isolate virus from PBLs of the SIV_{MAC}-

infected animals by H9 co-cultivation during rsCD4 treatment did not mean that these animals were cleared of virus. If $CD8^+$ lymphocyte-depleted rhesus monkey PBLs are co-cultivated with human PBLs in IL-2-supplemented medium, low levels of virus infection can be detected[18]. Using this method we were able to isolate virus from these animals during treatment. Nevertheless virus was more difficult to isolate during treatment than before and after treatment, and this probably reflects a quantitative decrease in replication of SIV_{MAC} in these animals. The coincident improvement in bone marrow function during this period of treatment is a functional benefit probably related to the decreased virus load. This increase in bone marrow colony-forming cells was not seen in the treated normal animals. The unexpected protection of bone marrow cultures from treated normal animals against SIV_{MAC} infection suggests another therapeutic benefit of rsCD4 treatment.

It has been suggested that rsCD4 could act by absorbing soluble gp120 and thus interfering with cytolytic T lymphocyte killing of HIV-1 infected targets, or by inhibiting cell fusion, or by direct blocking of virus-target cell binding[19]. It could also act indirectly to provide a therapeutic benefit to HIV-1-infected individuals. Although we do not know the precise mechanism by which it works, the beneficial effect of the rsCD4 treatment in SIV_{MAC} -infected rhesus monkeys suggests that it could be of value in treatment of humans infected with HIV-1.

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Induction of endogenous IFN-α and IFN-β genes by a regulatory transcription factor, IRF-1

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Interferons (IFNs) have an important role in cell growth and differentiation. The most well-known function of IFNs is their antiviral activity; viral infections result in induction of the transcription of the IFN-α and IFN-β genes[1-3]. Recently we isolated the gene encoding a transcription factor, IRF-1, that may play a part in the induction of IFN genes[4,5]. Interestingly, the IRF-1 gene itself is virus-inducible, suggesting the importance of *de novo* production of IRF-1 in IFN gene induction[5]. Here we show that high-level expression of the cloned mouse IRF-1 gene in monkey COS cells results in the induction of endogeneous IFN-α and IFN-β genes without viral stimulation. Furthermore, we demonstrate the induction of these genes by an IRF-1/yeast GAL4 chimaeric transcription factor. This may be the first demonstration of the specific induction of silent chromosomal genes by transfection of a single transcription factor gene in mammalian cells.



Fig. 1 Expression of murine IRF-1 and its derivatives in COS cells. *a*, Structure of expression vectors. Numbers in parentheses indicate the amino-acid residues of each factor. In all constructs the genes were inserted into the expression vector CDM8, which contains the cytomegalovirus (CMV) promoter/enhancer and the SV40 *ori* sequences[13]. They were (1) pCMIRS, which contains the entire mouse IRF-1 coding sequence downstream of the CMV promoter/enhancer sequence in the sense orientation; (2) pCMIRA, which is the same as pCMIRS except that the IRF-1 gene is introduced in the antisense orientation; (3) pCMIRD, in which a translational terminator sequence has been introduced within the cDNA sequence of pCMIRS, resulting in a gene product that lacks the carboxyl-terminal portion of the IRF-1 (that is, downstream from amino acid 189); (4) pCMIRG, which encodes a chimaeric factor consisting of the amino-terminal portion of IRF-1 and the yeast GAL4 transcriptional activation domain[14]. *b*, Detection of DNA-binding activity in COS cell transfectants. DNA transfection was performed by a method modified from Seed and Aruffo[15]. COS cells in a 10-cm dish (5×10^6 cells per dish) were transfected in 5 ml Iscove's modified Eagle's medium containing 10% NuSerum, 500 $\mu g\ ml^{-1}$ DEAE dextran, 100 μM chloroquin diphosphate and 5 $\mu g\ ml^{-1}$ plasmid DNA. Transfection efficiency of this protocol is ~30%, as estimated by the Tac antigen (IL-2 receptor)-positive COS cell population after transfection of pSVIL2R-3 (ref. 16). Whole cell extracts of transfected COS cells with pCMIRA (lanes 2 and 3), pCMIRS (lanes 4 and 5), pCMIRD (lanes 6 and 7) and pCMIRG (lanes 8 and 9) were prepared 48 h after transfection[4]. Each extract was subjected to gel shift analysis using ^{32}P-labelled C13 oligomer (sequence AAGTGA repeated 3 times in tandem) as probe[6]. The binding mixture (10 μl) contains 20 mM HEPES, *p*H 7.6, 0.5 mM dithiothreitol, 0.1 mM EGTA, 1 mM $MgCl_2$, 4% Ficoll, herring sperm DNA (1 μg), poly(dI-dC)·poly(dI-dC) (2 μg), ^{32}P-probe (5 fmole) and 2 μg protein of whole-cell extract. In lanes 3, 5, 7 and 9, a molar excess of unlabelled C1 oligomer (AAGTGA repeated 4 times in tandem)[6] (5 pmole) is also included. Lane 1: no extract. A low-level of the monkey IRF-1-like activity can be seen in lane 2. The shifted bands migrating faster in lanes 4 and 8 might be breakdown products of IRF-1 which still retain the DNA-binding property of native IRF-1. The intensity of the shifted bands was about 8 (pCMIRS), 110 (pCMIRD) and 32 (pCMIRG) relative to the control (pCMIRA). Following viral induction, a twofold increase is observed in untransfected controls (data not shown).

In order to study the function of IRF-1 in IFN gene expression, we constructed a series of expression vectors for the mouse IRF-1 gene[5] (Fig. 1*a*). The expression vectors were each transfected into monkey COS cells and the gene products were analysed by gel retardation using cell extracts and a ^{32}P-labelled C13 oligomer probe[6]. As shown in Fig. 1*b*, a significant increase in DNA binding activity was observed in the transfectants pCMIRS, pCMIRD and pCMIRG, demonstrating that the expected products are in fact produced in large amounts (see legend).

We next tested whether the increased IRF-1 expression results in the induction of otherwise silent IFN genes. As shown in

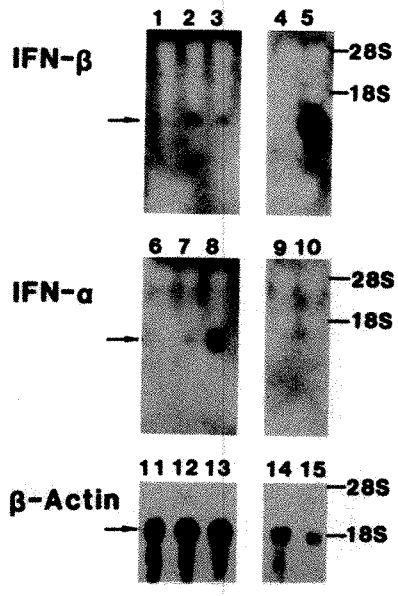


Fig. 2 Detection of IFN-β and IFN-αI specific messenger in COS cell transfectants. Twenty μg poly(A)$^+$ RNA from COS cells transfected with pCMIRA (lanes 1, 6 and 11), pCMIRS (lanes 2, 7 and 12) and pCMIRG (lanes 3, 8 and 13) and 10 μg total RNA from mock-infected COS cells (lanes 4, 9 and 14) and NDV-infected (10 h) COS cells (lanes 5, 10 and 15) were subjected to northern blotting analysis. It has been shown that cross-hybridization analysis of monkey genomic DNA with human IFN-α and IFN-β probes results in very similar patterns under stringent hybridization conditions[17]. Hybridization was with a ^{32}P-labelled human IFN-β probe (1.6-kilobase (kb) *Bam*HI-*Eco*RI fragment of pSE-66 (ref. 18), 8×10^8 c.p.m. μg^{-1}, lanes 1–5), human IFN-αI probe (0.7 kb HgiAI fragment of Hif-2h cDNA[19], 9.2×10^8 c.p.m. μg^{-1}, lanes 6–10) and human β-actin probe (2.0 kb *Bam*HI-*Pvu*II fragment of λHa-204 (ref. 5), 8×10^8 c.p.m. μg^{-1}, lanes 11–20). The filters were exposed at −80 °C for 2 days (IFN-α and IFN-β) or 4 h (actin). Densitometric measurement revealed the relative intensities of the bands in lanes 2, 3, 5, 7 and 8 as 1, 1, 259, 1 and 8.7, respectively. The apparent increase in IFN-α mRNA in lane 8 possibly represents induction of multiple IFN-α genes, the product of which might not have the same specific activity[2]. 28S and 18S ribosomal RNA size markers are shown to the right.

Table 1 IFN production in COS cells

COS cell supernatant	Interferon activity (U/5×10^6 cells) Experiment 1	2
Virus-induced cells		
Mock-induced (control)	<1	ND
NDV-induced*	5,120	ND
NDV-induced +anti-Namalwa-IFN†	5,120	ND
NDV-induced +anti-IFN-β‡	<1	ND
Antiviral activity in DNA-transfected cells		
pCMIRA	<1	<1
pCMIRS	160	160
pCMIRD	<1	<1
pCMIRG	320	230
pCMIRG+anti-Namalwa IFN	ND	80
pCMIRG+anti-IFN-αII§	ND	60
pCMIRG+anti-IFN-β	ND	110
pCMIRG+anti-Namalwa, αII and β	ND	<1
IFN-β specific ELISA‖		
pCMIRD	ND	<1
pCMIRG	ND	14

Values are standardized against reference human IFN-β. ND, not determined.

* Virus inactivated at *p*H 2.

† Anti-Namalwa IFN-α serum (polyclonal) (from Dr S. Yonehara, Tokyo Metropolitan Institute of Medical Science), final concentration 1%. The purified Namalwa-derived IFN used for the antibody preparation consists mainly of IFN-αI, but the presence of IFN-αII and IFN-β are not strictly ruled out (ref. 12; S. Yonehara, personal communication). Therefore, the IFN-αI level measured by the neutralization experiment could be an overestimation.

‡ Anti-human IFN-β serum (polyclonal) (from Dr T. Sudo, Biomaterial Institute, Tokyo), final concentration 2.5%.

§ Anti-recombinant human IFN-αII serum[7] (polyclonal) (from Dr G. Adolf, Ernst Boehringer Institut für Arzneimittelforschung, Vienna), final concentration 2.5%.

‖ Enzyme-linked immunosorbent assay (Toray, Tokyo). The value is likely to be an underestimation as the antibodies (the enzyme conjugate consists of a monoclonal antibody) are against human and not monkey IFN-β.

Table 1, antiviral activity was detectable in culture supernatants of the COS cells transfected with pCMIRS and pCMIRG but not with pCMIRA and pCMIRD. The detected IFN activity was attributable to IFN-αI, IFN-αII (also referred to as IFN-ω; ref. 7) and IFN-β. The IFN-β production was also confirmed by enzyme-linked immunosorbent assay. A combination of antibodies specific for human IFN-αI, αII and β completely neutralized the antiviral activity, implying that type II IFN (IFN-γ) was not generated using the transfectants. In contrast, COS cells infected with Newcastle disease virus (NDV) produced primarily IFN-β (Table 1). Induction of IFN mRNA was analysed by northern blotting. Both IFN-α and IFN-β messenger RNAs were detected specifically in the pCMIRS- and pCMIRG-, but not in pCMIRA- and pCMIRD-transfected COS cells. Although IRF-1 displays a lower affinity for IFN-αI compared with IFN-β (ref. 5), comparable levels of mRNA were induced. The overall IFN-α mRNA may be a reflection of the multiple subtypes induced[2]. The levels of IFN-β mRNA were much lower when compared with NDV-induced COS cells, but the mRNA size was the same in all samples, suggesting that mRNA initiation had occurred at the genuine CAP site of each gene. Similar results were obtained by expressing a complementary DNA encoding the human IRF-1 (data not shown). From the data, it seems that the amino-terminal region of IRF-1 is responsible for recognition of the regulatory DNA sequences, whereas the carboxyl-terminal region participates in activating transcription. IFN-α mRNA seems to be preferentially induced by the chimaeric factor (Fig. 2).

Two interesting issues arise from these observations: (1) Why is the IFN-β expression level much lower in the transfected cells compared with the NDV-induced cells? (2) Why are IFN-α genes induced by transfecting the IRF-1 cDNA but not by NDV in COS cells? In addressing the first question, it is possible that the IFN-β gene is subject to control mechanisms(s) that can be relieved upon viral induction[4,8–10]. In the IRF-1 gene-transfected cells, IRF-1 produced in large amounts might overcome such a control mechanism, resulting in activation of transcription, albeit at a low level. Alternatively, an additional signal(s) that can be delivered following viral infection may be needed[4,6]. In fact, NDV induction of pCMIRS-transfected COS cells resulted in about a 3- and 4-fold increase in induced IFN-β and IFN-α mRNA respectively (data not shown). These possibilities are not mutually exclusive. As for the second question, the molecular events causing this interesting difference are unclear at present. It is most likely that IRF-1 promotes transcription of IFN-α genes by interacting with their regulatory elements, because many, if not all, of the genes contain the elements recognized by IRF-1[4,5,10]. It is possible that the factor binds to those elements by displacing proteins (either specific factors or non-specific proteins such as histones) bound to chromosomal IFN-α genes. In cells that can naturally produce IFN-α, for example monocytes or B cells[1,2,11], the chromosomal structures might be

different from IFN-β. The results presented in this study provide strong evidence that the IRF-1 regulates the expression of type I IFN genes.

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Phosphorylcholine acts as a Ca^{2+}-dependent receptor molecule for lymphocyte perforin

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Large granular lymphocytes and cytolytic T-lymphocytes (CTL) contain numerous cytoplasmic granules thought to be responsible, at least in part, for the cytolytic activity of these effector cells. Isolated granules are lytic for a variety of target cells and the granule proteins are specifically released upon target-cell interaction[1-4]. Major proteins in mouse CTL granules are a family of seven serine proteases designated granzymes A to G[5], and a pore-forming protein called perforin (cytolysin)[1-4,6]. Purified perforin is cytolytic in the presence of Ca^{2+} and shows ultrastructural, immunological and amino-acid sequence similarities to complement component C9[6-8]. Despite these similarities, perforin and C9 are clearly distinct in their mode of target-cell recognition. Whereas C9 insertion is absolutely dependent on a receptor moiety assembled from the complement proteins C5b, C6, C7, and C8[9] on the target-cell membrane, no requirement for a receptor molecule has been reported for perforin. Here, we demonstrate that phosphorylcholine acts as a specific, Ca^{2+}-dependent receptor molecule for perforin.

Although complement component C9, in the absence of the C5b-8 receptor complex, inserts into and lyses small unilamellar vesicles[9], large multilamellar vesicles or membranes of erythrocytes are refractory to this C9 attack[10]. C9 is only able to intercalate non-specifically into the lipid bilayer without a receptor molecule if the density of lipid headgroups is low, as in the case of highly curved small vesicles. Similarly, perforin lyses small vesicles or other types of unilamellar vesicles without any apparent lipid specificity or need for a proteinaceous receptor molecule[2,11,12]. To exclude the possibility of non-specific insertion into this class of vesicles however, large multilamellar vesicles were tested for their inhibitory activity of perforin-mediated cytolysis. It has been shown that vesicles or lipoproteins compete with the red blood cell membrane for the binding of perforin and, hence, reduce the activity of perforin in a haemolytic assay[13-15]. Figure 1*a* shows that only vesicles containing lipids with phosphorylcholine headgroups such as sphingomyelin and phosphatidylcholine were inhibitory. This is consistent with the observation that perforin-mediated haemolysis is inhibited by 1 mM choline phosphate, whereas the presence of 50 mM ethanolamine phosphate, has no effect[15]. Moreover, phosphorylcholine-modified bovine serum albumin (BSA) was a potent inhibitor at 3 μg ml^{-1} (corresponding to approximately 1.3 μM phosphorylcholine molecules), whereas no competition of perforin-induced haemolysis was observed by non-modified BSA (Fig. 1*b*).

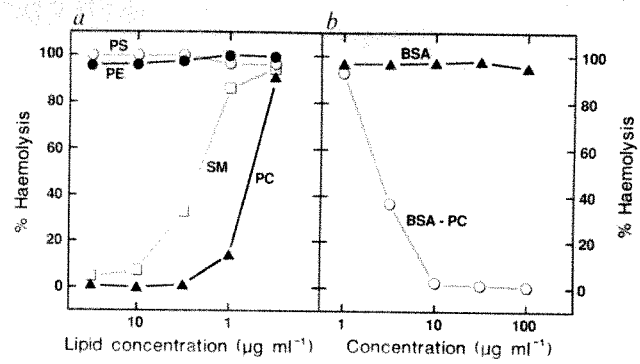


Fig. 1 Inhibition of perforin-mediated haemolysis by lipid vesicles. *a*, Inhibition of haemolysis by vesicles composed of phosphatidylcholine (PC, ▲), phosphatidylserine (PS, ○), phosphatidylethanolamine (PE, ●), sphingomyelin (SM, □), plus cholesterol in a 1:1 molar ratio. *b*, Inhibition of haemolysis by phosphorylcholine-BSA conjugates (BSA-PC, ○) or as control, by BSA (▲). Both phosphorylcholine and *p*-nitrophenylphosphorylcholine inhibited perforin's lytic activity at 1 mM (data not shown) indicating that the measured inhibition was indeed due to the phosphorylcholine moiety and not a result of the linker molecule used for its cross-linking to BSA.

Methods. Multilamellar liposomes of phospholipids and cholesterol were prepared by mixing 0.5 μmol of the phospholipid (Sigma) with 0.5 μmol of cholesterol in chloroform. The mixture was evaporated under reduced pressure to form a lipid film on the wall of a conical-bottomed flask. The dried lipid film was hydrated by vortexing at 50 °C in 0.1 ml of Tris-buffered saline (TBS, 10 mM Tris-HCl, *p*H 7.4, 150 mM NaCl). The liposome preparation was washed twice in the same buffer before use. Phosphorylcholine-conjugated BSA was prepared by incubating BSA (Fluka) with a 60-fold molar excess of *p*-diazonium phenylphosphorylcholine, synthesized as described[24], for 2 h at 20 °C in 0.035 M sodium borate buffer *p*H 9.2, containing 0.08 M NaCl. The excess reagent was removed by dialysis. Approximately 29 haptenic groups were bound per BSA molecule. The haemolytic activity of perforin was assessed as described[7]. Briefly, perforin (100 μl of a concentration that had been adjusted to lyse 80% of the sheep red blood cells) was mixed with 10 μl of liposomes or BSA and immediately added to sheep erythrocytes (1.5×10^7 cells ml^{-1}) in 300 μl veronal buffered saline (GVB, 10 mM veronal buffer *p*H 7.4, 142 mM NaCl, 0.1% gelatin, and 0.15 mM Mg^{2+}). The mixture was incubated for 15 min at 37 °C, the erythrocytes spun down and the degree of haemolysis measured by determining the haemoglobin release at 405 nm.

Direct evidence for perforin binding to phosphorylcholine was obtained by applying perforin or a CTL-granule extract (containing perforin and the granzyme lymphocyte proteases as major components) onto a nitrophenylphosphorylcholine-Sepharose column. At normal ionic strength and in the presence of 5 mM Ca^{2+}, perforin bound to the column (Fig. 2). At Ca^{2+} concentrations below 0.1 mM, perforin was not retained (Table 1). Once perforin had bound to the column, the removal of Ca^{2+} by washing the column with 'Ca^{2+}-free' buffer did not release perforin; it only eluted after the addition of 5 mM EDTA. Ca^{2+} could not be replaced by Mg^{2+} (Table 1). Binding of perforin

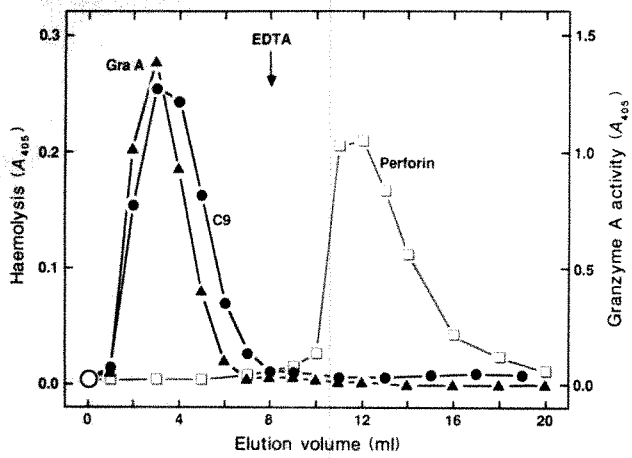


Fig. 2 Binding of perforin to a phosphorylcholine-Sepharose column. Purified perforin, an extract of granule proteins (perforin and granzyme A activity were determined) or C9 were passed over a phosphorylcholine-Sepharose column.
Methods. Phosphorylcholine affinity resin was prepared by reacting p-diazonium phenylphosphorylcholine with glycyltyrosine-conjugated Sepharose 4B as described previously[24]. Perforin and granule extracts were isolated from granules of the CTL line B6.1 (ref. 25). Granules were routinely isolated from 5×10^8 B6.1 cells. Cells were broken by nitrogen cavitation, and the organelles were separated on a discontinuous Percoll gradient. Soluble granule extracts were prepared by adding 1.5 M NaCl, thereby disrupting the granules[25]. Perforin or granule extracts were dialysed overnight at 4 °C against TBS containing no calcium. 100 μl aliquots were passed over a 2 ml phosphorylcholine-Sepharose column equilibrated in TBS containing 5 mM $CaCl_2$. Perforin was eluted with TBS containing 10 mM EDTA. Perforin activity was determined as described above. The esterolytic activity of granzyme A was assayed using benzoyl-lysine-thiobenzylester[5]. C9 activity was determined using C5b-8-bearing erythrocytes[7].

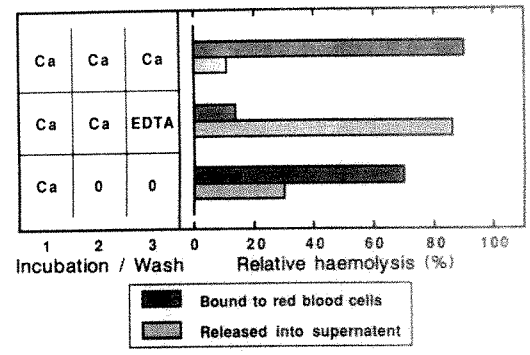


Fig. 3 Binding of perforin to red blood cells. Perforin was incubated in the presence of 5 mM Ca^{2+} with sheep red blood cells and washed and incubated: twice with a buffer containing 5 mM Ca^{2+}; once with 5 mM Ca^{2+} and once with 10 mM EDTA; or twice with 'Ca-free' buffer. The portion of perforin bound to the red blood cells (dark bars) or released into the supernatant (light bars) was determined after the last wash/incubation step.
Methods. 400 μl sheep red blood cells (1.5×10^7 cells ml^{-1}) in GVB containing 5 mM Ca^{2+} were incubated at 0 °C (on ice) during 10 min with a dilution of perforin or granule extracts which was three times higher than that required for 100% lysis at 37 °C after 15 min. No lysis occurred at this point. The cell suspensions were centrifuged and resuspended at 0 °C: twice in GVB containing 5 mM Ca^{2+}; once in GVB containing 5 mM Ca^{2+} and once in GVB containing 10 mM EDTA; and twice in GVB without Ca^{2+} ('Ca^{2+}-free' buffer). This last incubation was for 10 min on ice before the red blood cells were spun down. The pellet of red blood cells was then resuspended in 400 μl fresh GVB containing 5 mM Ca^{2+} and the mixture was incubated at 37 °C for 15 min. Perforin-induced haemolysis was determined as described in the Fig. 1 legend. The haemolytic activity of the unbound/released perforin was assayed by adding the supernatant after the last centrifugation step (300 μl) to 100 μl of concentrated sheep red blood cells (4.5×10^7 cells ml^{-1}) in GVB containing 5 mM Ca^{2+} (or 20 mM Ca^{2+} in the case when EDTA was used in the last wash). Haemolysis was assayed as above. The sum of lysis was arbitrarily considered as 100%.

to phosphorylcholine was also highly dependent on the *p*H, as perforin was retained only above *p*H 6. This is consistent with the *p*H dependence of perforin's lytic activity (Table 1). Neither other granule proteins, that is, granzymes, nor complement C9 bound to the phosphorylcholine-Sepharose (Fig. 2).

Perforin, like C9[16], has been shown to interact with membranes at 0 °C without consequent membrane insertion and lysis[13]. Perforin (or granule proteins) were incubated with red blood cells at 0 °C in the presence of 5 mM Ca^{2+} (Fig. 3). Erythrocytes were then washed twice in Ca^{2+}-containing ice-cold buffer to remove excess perforin and the subsequent increase in temperature to 37 °C led to haemolysis. In contrast, if perforin-bearing erythrocytes were washed in the presence of 10 mM EDTA, haemolysis did not occur. Perforin dissociated from the perforin-bearing erythrocyte membrane, as its activity was found in the supernatant after pelleting the intact erythrocytes. When erythrocytes were transferred into 'Ca^{2+}-free buffer', haemolysis still occurred.

These results indicate that perforin, upon exocytosis and in the presence of Ca^{2+}, binds to phosphorylcholine moieties of the target-cell membrane before lipid insertion and target-cell injury. The identification of the receptor is based on the similarity of the binding characteristics of perforin to phosphorylcholine-Sepharose and to red blood cells, respectively (Table 1). Only the binding, but not the lysis (insertion) step is dependent on the high Ca^{2+} concentrations required for perforin-mediated cytolysis[1,2,4,13]. It is possible that, within the granules, perforin may have endogenously-bound ions (as has been shown for C9[17]) which, upon exocytosis, allow the binding of perforin to the target cells. This may partly explain recent results showing that *in vivo* primed CTL require far less Ca^{2+} than purified perforin for their cytolytic activity[18,19], and that EDTA was unable to completely inhibit CTL-mediated cytolysis, when highly sensitive target cells were used[20,21].

It has been proposed that perforin and C9 have arisen from a common ancesteral gene[7,8]. A lytic protein whose receptor molecule is almost universally distributed on every cell type is potentially very dangerous for the organism. Thus, perforin is stored sequestered in cytoplasmic granules and is only released upon target-cell recognition at the site of close contact between CTL and target cell. In contrast, C9, which circulates in plasma, has lost its capacity to interact with phosphorylcholine and has become dependent on the nascent C5b-8 receptor complex for the proper target-cell recognition. The recent primary structure determination of perforin has shown that no overall homology

Table 1 Characteristics of perforin binding to phosphorylcholine-Sepharose and sheep red blood cells

Characteristics	Half-maximal binding/lysis: PC-Sepharose binding	Red blood cells: binding	Red blood cells: lysis
*p*H	6.5	6.5	6.5 (refs 13, 14)
$[Ca^{2+}]$	0.5 mM	0.5 mM	0.5 mM (refs 1, 4, 13)
$[Ca^{2+}]$*	<1 μM	NA	<1 μM
Mg^{2+}†	neg.	neg.	neg.

Experiments to investigate perforin binding to the phosphorylcholine (PC)-Sepharose or to the red blood cells, respectively were carried out as described in the legends to Figs 2 and 3, respectively, except that the *p*H or the metal ion conditions for the column or incubation buffer were varied. Bis-tris was used instead of Tris-HCl, when the *p*H was below 6.5. Concentration <1 μM stands for 'calcium-free' buffer. NA, not applicable; neg., negligible.
* After binding has occurred.
† 5 mM.

exists between perforin and C9[22,23]. The structural differences at the N and C termini may account for the functional dissimilarity.

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A kinetic intermediate in the reaction of an antigenic peptide and I-E^k

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Helper T cells are triggered by molecular complexes of antigenic peptides and cell surface glycoproteins of the MHC (gene products of the major histocompatibility complex) on antigen-presenting cells[1]. There is now a lot of evidence that the complexes between isolated class II MHC molecules and selected peptides have long half-lives of approximately one day[2,3]. The reported equilibrium binding constants between antigenic peptides and class II MHC molecules however, are only micromolar[4,5], suggesting that the association rate constants are very low. The only reported[2] association rate constant is for a chicken ovalbumin peptide (OVA323–339) binding to I-A^d, and is indeed remarkably low, about 1 litre per mole per second. Prompted by these unusual data, we have used the pigeon cytochrome-*c* peptide pCytc(88–104) and I-E^k reconstituted in planar lipid bilayers on glass slides to investigate further the kinetics of peptide–MHC reactions. We report the formation of two IE^k-pCytc peptide complexes. One complex has slow apparent association and dissociation kinetics, very similar to those reported previously for the chicken ovalbumin peptide and I-A^d. The second complex forms and dissociates about a hundred times more rapidly. The short-lived complex shows peptide–MHC specificity and is a kinetic intermediate in the formation of the long-lived complex; the long-lived complex is recognized by specific T-helper cells.

The helper-T-cell hybridoma 2B4 recognizes the class II MHC molecule, I-E^k and the 17 amino-acid C-terminal peptide of pigeon cytochrome-c, pCytc (88-104)[6]. This hybridoma is triggered to produce interleukin-2(IL^{-2}) when pCytc peptide is presented by I-E^k incorporated in planar lipid bilayers on solid supports[7]. Pigeon-Cytc labelled at the N terminus with fluorescein isothiocyanate FpCytc) binds to I-E^k incorporated into planar membranes. The two peptides, pCytc and FpCytc, are almost equally effective in triggering the 2B4 hybriodma (unpublished results). Two protocols were used to study the kinetics of FpCytc binding to I-E^k in planar membranes. In one, the fluorescence intensity attributed to FpCytc-I-E^k complexes was measured as a function of time. A reaction between FpCytc and I-E^k was essentially complete after ~100 seconds when the initial peptide concentration was 80 μM (see Fig. 1*a*). The binding measurements for times less than 100 s are reproducible but are of uncertain significance because of possible mixing artefacts. To test the kinetic order of the reaction a second protocol was used with series of incubations varying the incubation time (t) and peptide concentration (c) so that the product, (ct) was a constant (Fig. 1*b*). The data are not consistent with a single-step pseudo first-order reaction that goes to completion, as this should lead to a constant amount of complex when (ct) is kept constant.

Table 1 Specificity of the fast reaction between pigeon cytochrome c peptide (88-104) and I-E^k in planar bilayer membranes

Reactants	Photon counts s^{-1}
I-E^k + FpCytc	9,864 ± 625*
I-A^d + FpCytc	< 1,000*
Lipid + FpCytc	< 1,000*
I-E^k + α I-E^k, then FpCytc	1,100†
I-E^k + α I-A^k, then FpCytc	9,860†
I-E^k + pCytc, then FpCytc	1,300‡

*Incubation was with 80 μM FpCytec for 2 min.
† Preincubation with anti-MHC antibody was for 5 min, then washed and 80 μM FpCytc added.
‡ 80 μM pCytc was incubated with I-E^k in planar membranes for 2 min and FpCytc was then added for another 2 min, then washed with PBS.

The rapid reaction of fluorescent peptide and I-E^k was found to be specific, as (1) fluorescent OVA peptide did not bind to I-E^k, (2) preincubation with an antibody specific for I-E^k inhibited rapid binding of FpCytc to I-E^k whereas the non-specific antibody (anti-I-A^k) did not, and (3) preincubation of unlabelled pCytc with the I-E^k-containing planar membrane inhibited rapid binding of FpCytc peptide (see Tables 1, 2). The number of I-E^k molecules per unit area in the membrane (~3×10^{11} cm^{-2}) corresponds to the number of I-E^k molecules used in the preparation of the I-E^k-containing vesicles, and was confirmed using fluorescinated anti-I-E^k antibody. In the experiments reported here the number of fluorescent peptides bound at maximal binding exceeded 50% of the I-E^k in the planar membrane. In a number of other experiments the maximum fluorescent peptide binding was so low (~10%) that it precluded kinetic measurements, because of a reduced signal: background ratio.

In the cellular assay, we measured the extent of T-cell stimulation (IL-2 secretion) as a function of the number of preformed complexes. The number of preformed complexes was established by incubation time and peptide concentration. The planar membranes containing the resulting I-E^k-peptide complexes were then washed, and T cells added (Fig. 1*c*). Again, the

Table 2 Specificity of the kinetic intermediate complex of pigeon cytochrome *c* and I-E^k

Reactants	Photon counts s^{-1}
I-E^k + FpCytc	5,800
I-E^k + FpCytc (2 min) then pCytc*	3,800
I-E^k + FpCytc (1 min) then pCytc	1,600
I-E^k FpCytc (2 min) then OVA	4,600
I-E^k + FpCytc (1 min) then OVA	4,300

Experimental conditions as described in Fig. 1*a* legend.
* Unlabelled peptides were incubated for 2 min at a concentration of 100 μM.

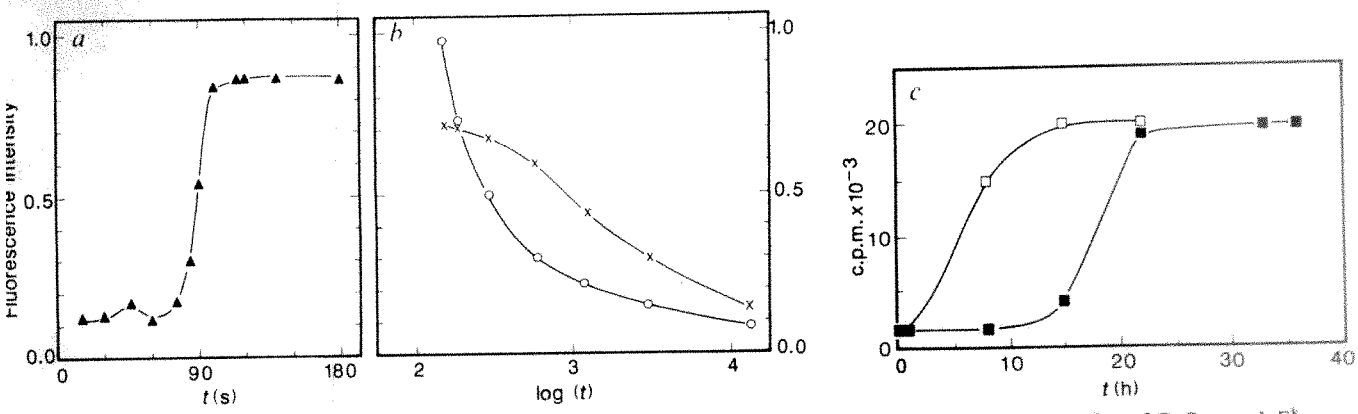


Fig. 1 Kinetics of binding of FpCytc to I-E^k in planar bilayer membranes. *a*, *b*, Fluorescence assays for the binding of FpCytc to I-E^k. *a*, Fixed 80 μM concentration of peptide versus incubation time. Fluorescence intensity is in units of 10^4 photons s^{-1}. Data are corrected for non-specific background, which is 30–40% of total signal. *b*, Fluorescence intensity is relative to the peak intensity. ○, FpCytc at concentration *c* incubated for time *t*, such that $tc = 12 \times 10^3$ μM s. X indicates theoretical points based[15], on estimated values of the rate constants k_{oi}, k_{io}, k_{ti}, k_{io} given in the text, using $k_{it} = 4 \times 10^{-5}$ s^{-1}. The values of X give the sum of the concentrations of C_i and C_t. The maximum value of X corresponds to 71% occupation of the available binding sites. A larger value of $k_{it} = 4 \times 10^{-4}$ significantly increases $(C_i + C_t)$ only at the two longest times. *c*, Cellular assay for the kinetics of binding of pCyte to I-E^k. Solutions of 3 μM (■) or 30 μM (□) pCytc were incubated with I-E^k in planar membranes for the indicated times, the membranes washed, 2B4 T cells added, and IL-2 secretion was assayed by [^{3}H]thymidine uptake of the CTLL-2 cell line. The ratio of the two times (5.5 and 18 h) for 50% triggering is used to estimate the equilibrium dissociation constant K_i for the intermediate complex. The absolute value of each half-time can be used to estimate the rate constant k_{it} for the unimolecular transition of the intermediate complex to the terminal complex under the assumption that 50% of maximal T-cell triggering corresponds to 50% occupation of available I-E^k peptide binding sites. Competition experiments show that the half times in these experiments do not depend on the number of available I-E^k binding sites (unpublished results). This analysis is equivalent to drawing first-order binding curves through the mid-points of the experimental data, and assuming that the deviation of the data from these curves is due to non-linearity in T-cell responses. **Methods.** I-E^k was purified by affinity chromatography using monoclonal antibody 14-4-4S as described[9,10]. Lipopolysaccharide (*Escherichia coli* 0.111:84, Difco) stimulated blast cells from spleens of CBA (H-2^k) mice were used as the source of I-E^k. Reconstitution of I-E^k into planar membranes was as described[10]. Vesicle preparations for cellular assays used the lipid mixture of dipalmitoylphosphatidylcholine and dilinoleoyl phosphatidylcholine and cholesterol[7]. In *a*, 80 μM FpCytc was incubated with planar membranes containing I-E^k for the times indicated, then washed for one minute with PBS, all at room temperature (20 °C). Fluorescence intensity was measured with the photon counting system described previously[8]. The incubations were carried out in chambers of 20 μl volume assembled on a quartz microscope slide and covered by a circular 12-mm diameter coverglass using two layers of double sticky tape. Each microscope slide had two chambers. Vesicle preparations with or without I-E^k were deposited in the chambers, allowing planar membrane formation. N-terminal fluorescination of pCytc (88–104) was carried out during solid phase peptide synthesis (Peninsula), and purified by reverse phase HPLC on a Vydac C_{18} column. In *c*, the cell-free reaction took place during time intervals shown. Excess unbound peptide was removed by careful replacement of the medium covering the planar membrane, over a period of 10 min or more. T cells were added at a density of 4×10^5 to every well. The amount of IL-2 secreted in culture wells was assayed by [^{3}H]thymidine uptake of an IL-2-dependent T-cell line CTLL-2.

binding kinetics cannot be accounted for by a single-step pseudo first-order reaction. That is, the times for 50% cell triggering are not inversely proportional to the peptide concentration.

The experiments in Fig. 2 (▲) show the dissociation of the FpCytc-I-E^k complex as a function of time. FpCytc and I-E^k in planar membranes were incubated at room temperature for various lengths of time and were then sequentially rinsed to remove dissociated FpCytc. The wash was repeated at the indicated times for 2.5 days. After many washes the samples lose only 5–10% of the I-E^k as judged by the binding of anti-I-E^k. The dissociation curve is evidently biphasic with fast and slow components. Both reactions follow first-order kinetics with half lives of approximately 10 minutes and of 30 hours. The half life for the dissociation of pCytc from I-E^k at 37 °C obtained from the cellular assay is similar: 34 hours (Fig. 2, ●).

The dissociation data can be understood semiquantitatively in terms of two complexes of FpCytc and I-E^k, a short-lived intermediate complex C_i and a long-lived terminal complex C_t.

$$\text{FpCytc} + \text{I-E}^k \underset{k_{io}}{\overset{k_{oi}}{\rightleftharpoons}} C_i \underset{k_{ti}}{\overset{k_{it}}{\rightleftharpoons}} C_t \qquad (1)$$

The first-order dissociation rate constant for the intermediate of FpCytc complex in Fig. 2*a* is $k_{io} = 1.2 \times 10^{-3}$ s^{-1}. The first-order dissociation rate constant for the long-lived terminal complex is $k_{to} \approx k_{ti} = 6.3 \times 10^{-6}$ s^{-1}. The relative amplitudes of the slow and fast components of dissociation depend on the time of preincubation before the dissociation measurement. The longer incubation time gives a larger proportion of the slowly dissociating component. Thus a 5-min incubation gives ~15% of the slow component, whereas a 20-min incubation gives ~30% of the slow component, both with 80 μM FpCytc.

These results can be interpreted using a first-order rate constant for the conversion C_i to C_t of $k_{it} = 4 \times 10^{-4}$ s^{-1}. The data in both Fig. 1*c* and Fig. 2 provide evidence that C_i is an intermediate in the formation of C_t and is not some irrelevant dead-end complex. The experiments do not show whether C_i is a complex with only one bound peptide (FpCytc) or two peptides (for example, FpCytc and a second peptide). The slowly decaying component C_t can be identified as biologically active, given the excellent agreement of the low off-rates based on the fluorescence and cellular assays (Fig. 2, ●).

According to reaction (1) the resultant kinetics of the formation of the terminal complex C_t from peptide and I-E^k are pseudo first-order with a rate constant that is non-linear in the peptide concentration, $k_{ot} = (k_{it})/(p + K_i)$, where p is the peptide concentration and K_i is the dissociation constant of the intermediate complex C_i. The data in Fig. 1*c* can be interpreted with $K_i \approx 10^{-5}$ M and $k_{it} \approx 4 \times 10^{-5}$ s^{-1}; this value for k_{it} assumes the cellular response is half-maximal at 50% saturation of the I-E^k with pCytc. The disparity between the two estimates of k_{it} is not unreasonable in view of the uncertainties in this assumption. We estimate the equilibrium dissociation constant K_t of the terminal complex from the equation $K_t = (k_{ti}/k_{it})K_i$ and obtain $K_t \sim 10^{-6}$–10^{-7} M, depending on the choice of k_{it} from the above estimates. Complexes of other antigenic peptides and Ia studied by Babbitt and co-workers[4] and Buus and co-workers[5] have dissociation constants $K_t \sim 10^{-6}$ M. Our model reduces mathematically to that of Buus at low peptide concentrations ($p \ll K_i$) where only three constants are observed, K_t, k_{ot} and k_{to}.

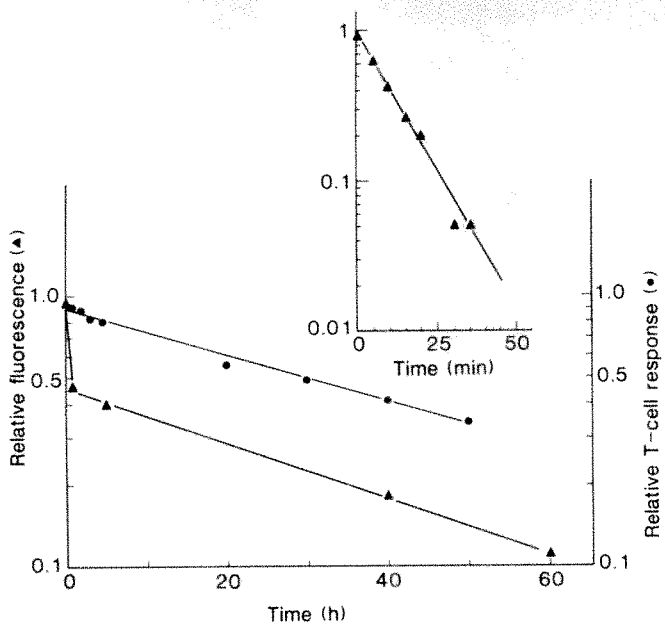


Fig. 2 Kinetics of dissociation of pigeon cytochrome-*c* peptide (88–104)-I-E^k complex. Fluorescence assay, ▲, curve and insert). Following an ~25 min incubation of I-E^k in planar membrane together with 80 μM FpCytc, the sample was washed sequentially at the indicated times. Each wash lasted about 1 min, and involved flowing 10–15 volumes PBS through the sample chamber. The data given in the insert were obtained after a 5-min incubation. Fluorescence intensities in the insert are used to estimate the off-rate constant k_{io} for the intermediate complex, and are corrected for both background fluorescence and the fluorescence intensity of the long-lived complex. After this 5-min incubation the short lived fluorescence (10-min half-life) is about 85% of the total specific fluorescence. The half-time for the dissociation of FpCytc from the terminal complex from the fluorescence data is 30 hours. In the cellular assay, (●) 15–20 μM unlabelled peptide was incubated at 37 °C with planar membranes with I-E^k for 24 h in RPMI medium without serum. Excess unbound peptide was removed. Each data point was obtained by allowing dissociation of peptide from I-E^k–peptide complexes followed by a second rinse before addition of 2B4 T cells in 10% fetal calf serum. The half time obtained from these cell assay data is 34 h. At each time point, the proliferation of CTLL was measured by titrating the supernatant and using data in the linear range.

The kinetic scheme (1) does not account for the observed but uncertain shape of the short time (<100 s) binding curve. A value of $k_{oi} = 1.2 \times 10^2$ l mol^{-1} s^{-1} obtained from the above estimates of K_i and k_{io} leads to a half-time for the formation of C_i of 70 seconds at 80 μM peptide, a result close to the onset time of ~90 seconds seen in Fig. 1*a*. These estimated values of K_i and k_{oi} also lead to the calculated points X in Fig. 1*b*, to be compared with the experimental points O. The calculations used the solution for two sequential first-order reactions given by Moore and Pearson[15]. At short times and high peptide concentrations the fraction of the available I-E^k binding sites occupied by the peptide is approximately $pk_{oi}k_{ii}t^2$, so that even at times as short as 100 s as much as 1% terminal complex can form, which may account for some of the very recent observations on the kinetics of peptide–MHC complex formation, as measured by calcium influx in specific T cells[16].

In summary, a variety of kinetic data all point to the formation of an intermediate complex between an antigenic peptide and an MHC molecule. This result is particularly significant as the intermediate complex shows peptide-MHC specificity. The molecular structures of the intermediate and terminal complexes may be very different as the time required for the unimolecular conversion of the intermediate complex to the terminal complex is long, estimated to lie between 30 and 300 min. The slow step may or may not involve the release of a second peptide. Thus, although specific helper T cells presumably recognize the structure of the terminal complex, the selective peptide recognition by MHC may involve a substantially distinct protein-peptide conformation[12–14].

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Identification of specific binding proteins for a nuclear location sequence

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The nuclear envelope is a selective barrier against the movement of macromolecules between the nucleus and cytoplasm[1]. Nuclear proteins larger than relative molecular mass 20,000–40,000 are probably actively transported across the envelope through the nuclear pore complex[2,3] and are directed by specific nuclear location sequences (NLS) in the proteins[4]. NLS mediate the nuclear import of isolated nuclear proteins after microinjection into whole cells[5,6] and the nuclear accumulation of chimaeric proteins[7–9] or of non-nuclear proteins conjugated to synthetic peptides[10–12]. The best-characterized NLS is the simian virus 40 large T-antigen sequence[4,13,14]. We have identified two proteins of rat liver by chemical cross-linking that interact with a synthetic peptide containing this sequence: this interaction is specific for a functional NLS, is saturatable, and high affinity. The binding proteins are present in a post-mitochondrial supernatant, in nuclei and in a nuclear envelope fraction, which is consistent with a role in the transport of nuclear proteins from the cytoplasm to the nucleus.

Chemical cross-linking with disuccinimidyl suberate of a radioiodinated synthetic 34-residue peptide from the SV40 large T antigen containing the NLS was used to identify the proteins in different subcellular fractions of rat liver that interact with the NLS (Fig. 1). A 10-residue synthetic peptide containing the wild-type NLS sequence was used as a competitor to define binding specificity. Two proteins that interact specifically with the NLS, and can be competed out by a 100-fold molar excess of the 10-residue wild-type peptide, are detected in a post-

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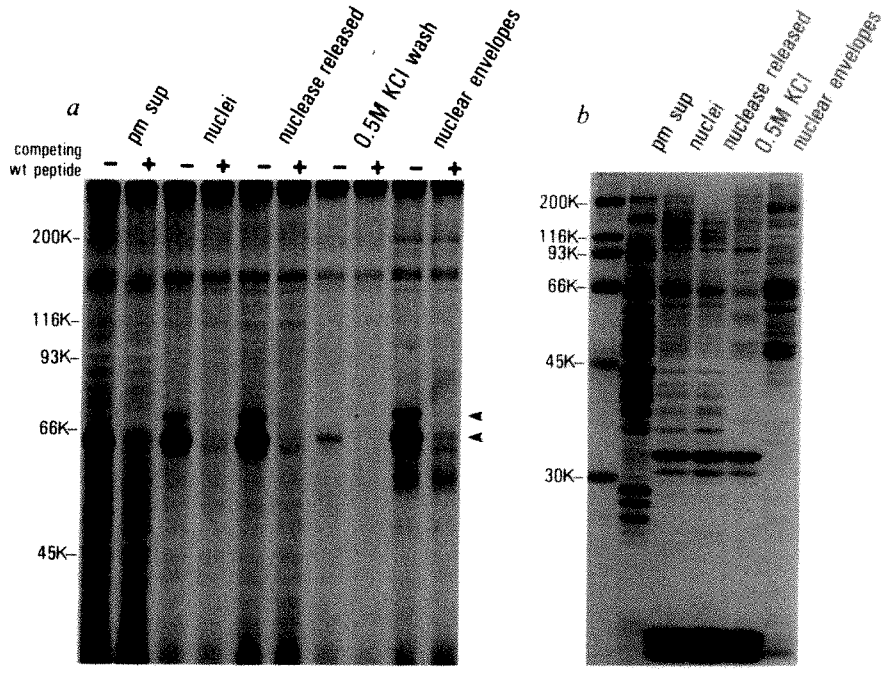


Fig. 1 Intracellular distribution and specificity of the SV40 large T antigen nuclear location sequence binding proteins. *a*, Equivalent amounts of protein from octylglucopyranoside/high salt extracts of post-mitochondrial supernatant (pm sup, 0.05 nuclear equivalents (n.e.) where 1 n.e. = 3×10^6 nuclei), whole nuclei (0.68 n.e.), nuclease-released soluble (1 n.e.), 0.5M KCl-wash (8.7 n.e.) and nuclear envelopes (14 n.e.) were incubated with radiolabelled peptide and cross-linked with disuccinimidyl suberate (Pierce). An autoradiograph of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel is shown. Arrows indicate specific bands. *b*, Coomassie-blue-stained gel of the subcellular fractions used in *a*. The lower portion of the figure presents the sequences of peptides used for cross-linking and competition. The underlined residues represent the minimum SV40 large T antigen nuclear location sequence. The 34-residue peptide was labelled with ^{125}I. Molecular weights of marker proteins are indicated.

Methods. Rat liver nuclear envelopes were prepared as described[17]. Post-mitochondrial supernatant was prepared by centrifugation of the first post-nuclear supernatant at 12,500*g* for 15 min. Portions of each fraction containing 50 μg protein were incubated in 20 μl buffer A (15 mM HEPES-KOH, *p*H 7.3, 0.25 mM $MgCl_2$, 8.5% sucrose, 1 μg ml^{-1} each leupeptin and pepstatin A, 2 mM dithiothreitol, 10 u ml^{-1} aprotinin) 1% octyl-β-D-glucopyranoside (Calbiochem) and 300 mM KCl for 1 hour on ice. The extracts were diluted sixfold in buffer A and 50 nM ^{125}I-labelled SV40 large T antigen peptide or the 10-residue peptides was added to 5μM for competition. The 34-residue analogue was labelled with ^{125}I using Iodogen (Pierce) to a specific activity of $3\text{–}5 \times 10^5$ c.p.m. ng^{-1} peptide. After 30 min at 25 °C, the samples were cooled on ice for 5 min and disuccinimidyl suberate was added to 0.5 mM from a 50 mM stock in dimethylsulphoxide. Cross-linking was terminated after 15 minutes by precipitation with trichloracetic acid added to 10%; precipitates were collected by centrifugation, washed with 95% acetone and air-dried before resuspension in SDS-PAGE sample buffer. Samples were resolved in 8% SDS-PAGE gels[18]. The gel in *b* is 11% polyacrylamide, rather than 8%, which resolves the histones in the nuclear fractions. The gels were fixed and stained with Coomassie blue, destained and dried before autoradiography using Kodak XAR-5 film with an intensifying screen at −80 °C.

Labelled peptide

cys–tyr–asp–asp–glu–ala–thr–ala–asp–ser–gln–his–
ser–thr–pro–pro–lys–^{128}lys–lys–arg–lys–val–glu–
asp–pro–lys–asp–phe–glu–ser–glu–leu–leu–ser–NH_2

Competing peptide

Wild type cys–pro–lys–^{128}lys–lys–arg–lys–val–glu–asp–NH_2

mitochondrial supernatant, in nuclei and in a highly purified nuclear envelope fraction by the cross-linking procedure (see arrows in Fig. 1*a* and *b*): these are a predominant protein of relative molecular mass (M_r) 60,000 (60K) and a minor protein of 70K (these values include the mass of the cross-linked peptide). The 70K protein in the post-mitochondrial supernatant can be difficult to visualize (as in Fig. 1), but usually is evident (data not shown). Isolation of nuclear envelopes is by nuclease digestion of nuclei to give a nuclease-released fraction (Fig. 1), followed by extraction in 0.5M KCl to solubilize residual chromatin (0.5M KCl wash, Fig. 1). Most of the NLS-binding proteins are released from nuclei by nuclease digestion, which is consistent with a partially intranuclear location. In different experiments, however, we find that 10–30% of the nuclear-binding activity is retained in the nuclear envelope fraction in a salt-resistant fashion. This indicates that at least a fraction of the NLS-binding proteins are associated with the nuclear envelope. The activity detected in the post-mitochondrial supernatant fraction is probably not a result of release of the proteins from the nucleus during preparation, because isolated nuclei incubated at 4 °C for several hours in the homogenization buffer do not release the binding proteins (data not shown). The binding proteins in the post-mitochondrial supernatant are retained in a high-speed supernatant and therefore are not associated with any membranes (data not shown). Although these data suggest that the location for NLS-binding proteins is partially cytoplasmic, definitive localization requires antibodies specific to these proteins for immunocytochemistry.

To detect the NLS-binding proteins optimally in nuclear envelopes, it was necessary to extract them with 1% octyl-β-D-glucopyranoside/0.3M KCL before cross-linking (Fig. 2). Substantially more cross-linking of the peptide to the NLS-binding proteins was seen in the detergent-soluble fraction (Fig. 2,OCG/KCl in lane S) compared with intact nuclear envelopes (buffer), with essentially no detectable binding protein remaining in the insoluble lamina fraction (OCG/KCl, lane P). The reason for this binding 'latency' is unclear. As these detergent and salt conditions completely solubilize some nuclear pore complex proteins[15] (data not shown), it is possible that the masked binding activity represents NLS-binding proteins associated with endogenous transport ligands which become dissociated in the presence of detergent and high salt. In contrast to nuclear envelopes, treatment of the other subcellular fractions with detergent and salt resulted in only a small increase in the binding proteins detected (data not shown).

The 60K and 70K labelled proteins were present in the same relative abundance in all subcellular fractions, but their relationship is unknown. The association of the synthetic peptides with

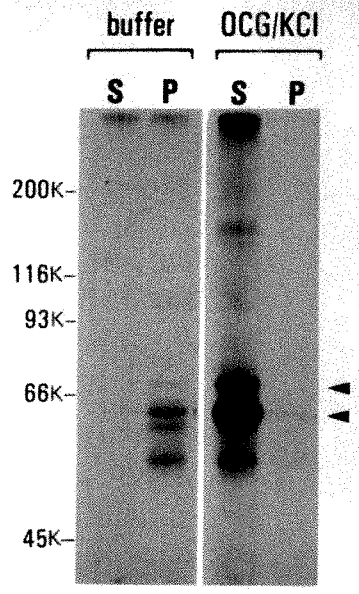


Fig. 2 Extraction of SV40 nuclear location sequence binding protein from nuclear envelopes. The binding proteins are masked in the nuclear envelope (buffer) but can be exposed by extraction in 1% octyl-β-D-glucopyranoside/0.3M KCl (OCG/KCl). Detergent-soluble (S) and insoluble material (P) after extraction and cross-linking are shown on an autoradiograph of the SDS-PAGE gel.

Methods. 50 μg nuclear envelopes were incubated in buffer A containing 50 mM KCl (buffer) or 1% OCG/300 mM KCl (OCG/KCl) as described in Fig. 1 legend. Soluble and insoluble fractions were separated after cross-linking by centrifugation at 5,000*g* for 15 min.

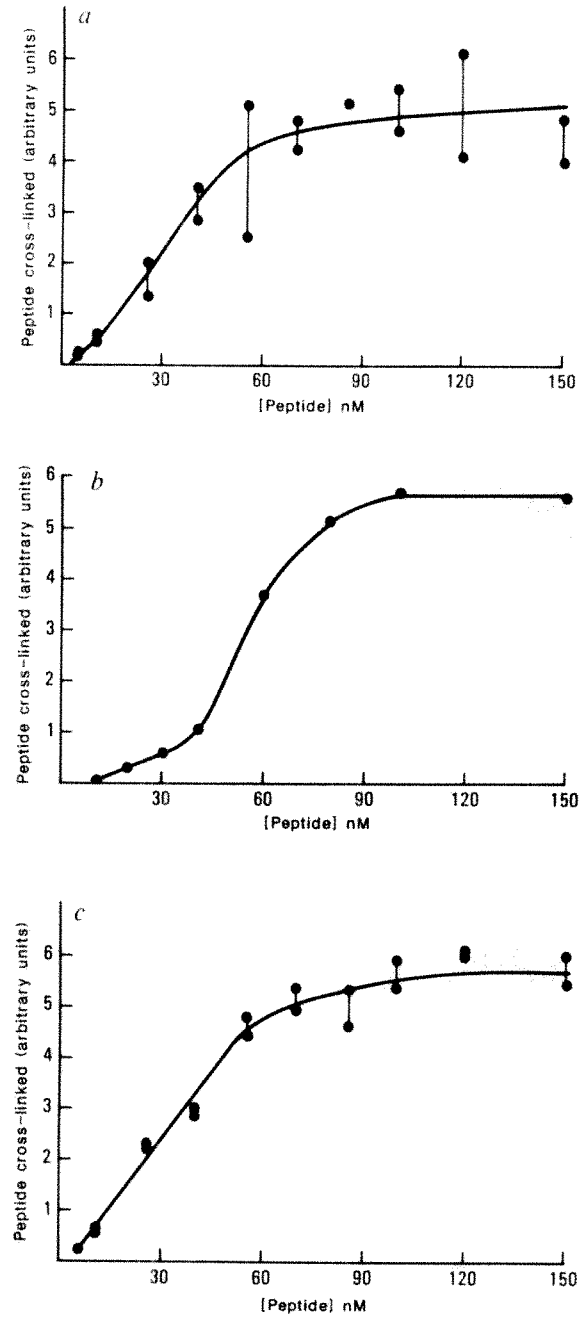


Fig. 3 Saturatable, high-affinity binding of the NLS peptide to the NLS-binding proteins. Cross-linking to (*a*) post-mitochondrial supernatant, (*b*) nuclease-released material or (*c*) nuclear envelopes demonstrates that the binding is saturatable and of high affinity. Data represent quantitation of the 60K protein; equal amounts of protein were analysed for each fraction.

Methods. Subcellular fractionation and cross-linking are as described in Fig. 1. Autoradiographs were scanned in an LKB Ultroscan XL laser densitometer. Peak areas were integrated automatically and used to plot the relative amount of radioactivity in each band. Arbitrary units were assigned to the nuclear envelope fraction and the values for the other fractions were adjusted to give similar values at saturation. Samples were run in duplicate, with the exception of the nuclease-released fraction which was determined once at each peptide concentration. Cutting individual bands from the gels and counting in a gamma counter gave similar results.

the binding proteins occurs rapidly, reaching equilibrium within minutes at 25 °C, and does not require ATP (data not shown). The binding proteins associated with the solubilized nuclear envelope (Fig. 2) are not immunoadsorbed with monoclonal antibodies to nuclear pore complex glycoproteins[15] and are therefore distinct from them (data not shown).

We used the peptide cross-linking assay to test the concentration dependence of the interaction of the 60K protein with the 34-residue peptide (Fig. 3). Saturation was achieved in all fractions at 50–75 nM peptide concentrations, which indicated a high-affinity interaction. The binding curves for the post-mitochondrial supernatant (Fig. 3*a*) and the nuclear envelope (Fig. 3*c*) material are hyperbolic, reaching half-maximal saturation at 30 nM peptide, whereas the nuclease-released (Fig. 3*b*) material gives a sigmoidal curve, with half-maximal saturation at almost 60 nM peptide. Whether this represents a true difference in binding properties or results from other differences between the fractions is unknown.

The specificity of binding was further analysed by competition with peptides containing sequences previously characterized by their ability to direct nuclear transport *in vivo*[14,16]. A 10-fold and 100-fold molar excess of each peptide was used to compete with specific binding of the 34-residue NLS peptide (Fig. 1*c*) in the cross-linking assay. A peptide containing the wild-type NLS (Fig. 4, wild type), as well as a peptide containing a mutation that does not affect transport *in vivo* (peptide A) strongly compete for binding with the NLS peptide. A peptide containing a mutation that abolishes transport *in vivo* (peptide B) does not inhibit binding, whereas two peptides containing sequences that allow partial transport compete weakly or not at all (peptides C and D). The observed competition with peptides containing transport-competent sequences is not a simple consequence of charge density on the peptides, as a peptide containing the reverse of the wild-type sequence (that is, from

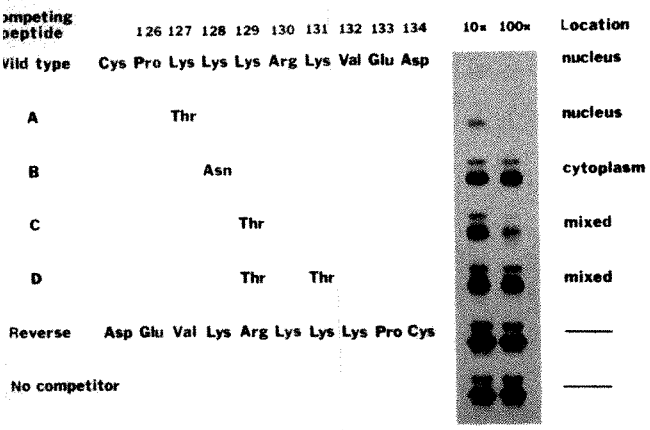


Fig. 4 Specific binding of the NLS peptide to the NLS-binding proteins. Cross-linking was as described in Fig. 1, using binding proteins extracted from nuclear envelopes. Cross-linking in the presence of 10-fold and 100-fold molar excess of competing peptides is shown. Subcellular locations were taken from ref. 14.

Methods. Wild-type, B and reverse peptides, as well as the 34-residue peptide, were synthesized by the stepwise coupling of activated protected amino acids by the solid-phase peptide synthesis method[19] on to 4-methylbenzhydrylamine resin using an Applied Biosystems 430A automated peptide synthesizer. The peptides were cleaved from the resin by liquid HF at 0 °C for 1 hour in the presence of anisole[19]. The 34-residue analogue was cleaved by the low/high HF method[20], modified to include cysteine-HCl. The crude peptides were purified by HPLC on Vydac RP-C18 or RP-C4 preparative columns using a water/acetonitrile-containing 0.1% trifluoroacetic acid (gradient 100/0 to 50/50). Peptides A, C and D were synthesized by the simultaneous multiple-peptide synthesis method[21] and were not further purified for this analysis. All peptides were further purified by chromatography on Sephadex G-10 in 0.1M acetic acid and concentrations of each were determined by Ellman's reaction[22].

arboxyl to amino residues) does not compete for binding (peptide reverse). Thus there is a close correspondence between the bility of different sequences to direct transport *in vivo* and their bility to compete with NLS-peptide binding to the 60K and 0K proteins *in vitro*.

These cross-linking experiments identify only two proteins in at liver that specifically interact with the SV40 large T antigen uclear location sequence. As the interaction is also high-affinity nd saturatable, it is most likely that the NLS-binding proteins ve have detected represent carrier molecules for nuclear import f proteins containing a functional SV40 large T-antigen NLS. he apparent distribution of these proteins in the cytoplasm nd nuclear interior as well as in the nuclear envelope supports multistep model for carrier-mediated nuclear protein import. he initial step, binding of the protein to be transported to a eceptor in the cytoplasm, could occur very soon after translaion; this receptor–protein complex could then be recognized t the nuclear pore complex by another receptor molecule and e translocated through the pore channel, concomitantly with hannel gating[3]. The internalized complex could then dissociate, vith the free receptor being recycled to the cytoplasm or legraded. Further characterization of the NLS-binding proteins lescribed here in conjunction with *in vitro* and whole-cell uclear transport systems will address some of these possibilities.

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Note added in proof: The reverse sequence peptide in Fig. 4 has ecently been shown to be incapable of directing nuclear transport *in vivo* (T. J. Lobl, personal communication).

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Role of transcriptional interference in the *Drosophila melanogaster* *Adh* promoter switch

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The *Drosophilia melanogaster* alcohol dehydrogenase (*Adh*) gene is transcribed from two closely linked promoters[1,2], which are regulated by two upstream enhancers. The proximal promoter is active primarily in first to early third-instar larvae, whereas the distal promoter is active in late third-instar larvae and adults (Fig. 1). The *Adh* larval enhancer and the proximal promoter are separated by the *Adh* adult enhancer and the distal promoter. Because the proximal promoter is turned off just as the distal promoter is turned on, we considered the possibility that the distal promoter or adult enhancer has a role in the downregulation of the proximal promoter. We report here that transcription from the distal promoter is required to shut off the proximal promoter. In the absence of the distal promoter, the proximal promoter is active throughout larval development and in adults. The proximal promoter is also aberrantly active in adults when placed upstream of the distal promoter. These results suggest that the developmental switch from proximal to distal promoter is regulated by the stage-specific activation of the distal promoter, and the subsequent repression of the proximal promoter by transcriptional interference[3–8].

Initially we deleted the distal promoter and the *Adh* adult enhancer (AAE) and examined the activity of the proximal promoter at various developmental stages. When the distal promoter and AAE were deleted (Fig. 2) the proximal promoter was not shut off in mid third-instar larvae but, rather, remained active throughout larval development (Fig. 3*a*, compare lanes 4–6 with 11–14). In addition, the proximal promoter was expressed at aberrantly high levels in adults (Fig. 3*a*, compare lanes 7 and 15). Thus, the distal promoter and AAE are required for the normal downregulation of the proximal promoter in third-instar larvae and in adults.

A deletion that removes the TATA box and initiation site of the distal promoter (−66 to +53), also leads to the derepression of the proximal promoter in adults (Fig. 4*a*, lanes 12–14). This result suggests that readthrough transcription from the upstream, distal promoter inactivates the proximal promoter (transcriptional interference[3–8]). Unlike other systems where transcrip-

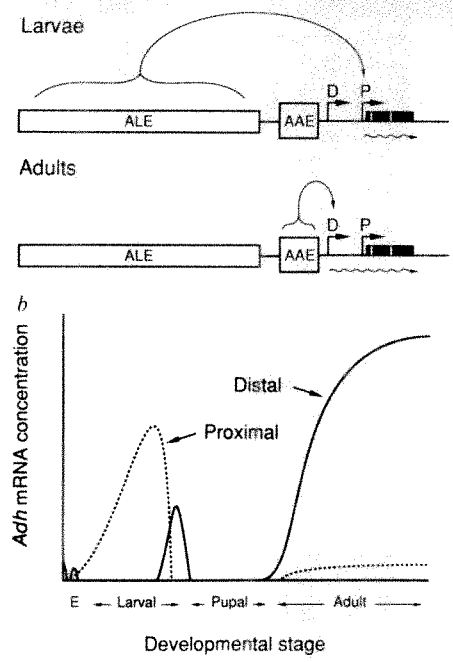


Fig. 1 The organization of *Adh* regulatory sequences and the pattern of *Adh* expression during development. *a*, The *D. melanogaster Adh* gene, showing the enhancer-promoter interactions in different developmental stages. In larvae, the *Adh* larval enhancer (ALE) stimulates transcription primarily from the proximal promoter (V.C. and T.M., manuscript in preparation). ALE activity maps to three disparate regions which lie between −5,000 and −660 base pairs (bp) of the distal promoter (V.C. and T.M., unpublished results). In adults, the *Adh* adult enhancer (AAE) stimulates transcription from the distal promoter[13]. The AAE is located between −590 and −128 bp of the distal promoter (D. Falb and T.M., unpublished observations). *b*, The relative levels of proximal (dotted line) and distal (smooth line) promoter transcripts are plotted as a function of developmental stage (taken from ref. 2).

tional interference has been detected[4,5], deletions that reduced the activity of the upstream (distal) *Adh* promoter, did not proportionately increase the activity of the downstream (proximal) promoter (Fig. 4*a*, lanes 6–11). This observation does not, however, eliminate the transcriptional interference model, as the levels of transcription from the weakened distal promoters are still relatively high and could therefore be sufficient to inactivate the proximal promoter.

To test whether the proximal promoter is repressed in adults by transcriptional interference, we placed a second copy of the proximal promoter and *Adh*-coding region upstream of the AAE and distal promoter, so that transcription from the distal promoter could not read through the upstream proximal promoter (5′*P*3′*D*P, Fig. 2). In this context, the proximal promoter was highly active in adults, even though the distal promoter was expressed at the usual high level (Fig. 4*b*, lanes 7–9). Thus, when transcription from the distal promoter cannot read through the proximal promoter, the proximal promoter is not shut off in adults.

These results are consistent with the transcriptional interference model, but they do not exclude the possibility that the distal promoter, by virtue of its location, blocks interactions between the proximal promoter and the *Adh* larval enhancer (ALE) or the AAE (see Fig. 1*a*; although the AAE does not normally stimulate the proximal promoter, it can if the distal promoter is deleted[13]). We tested this possibility by placing the ALE or AAE at the 3′ end of the gene, so that the distal promoter does not lie between the enhancer and the proximal promoter. The stage-specific expression of the two promoters was not altered when the ALE was placed downstream from the gene (V.C. and T.M., manuscript in preparation). Similarly, the proximal promoter was repressed normally in adults when the AAE was placed downstream from an *Adh* gene containing both promoters. (Fig. 4*b*, lanes 5 and 6). In contrast, the 3′AAE did activate the proximal promoter in adults when the distal promoter was deleted (data not shown). We conclude that the stage-specific activity of the proximal promoter is independent

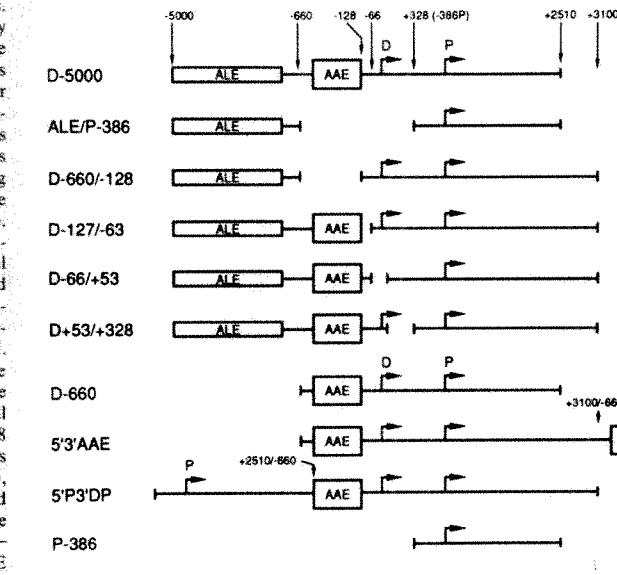


Fig. 2 Structure and expression of mutant *Adh* genes. The structure (not to scale) and transcriptional activity of *Adh* gene constructs in adult transformants are shown. In wild-type adults, the distal promoter is active at high levels (+) and the proximal promoter is active at low levels (−). Different levels of transcripts were observed with independent transformants containing the same *Adh* mutants. This variability is presumably due to the influence of surrounding chromosomal sequences or structure[14]. Therefore, we have indicated the activity of a given gene as a range. N/A, not applicable. Deletion endpoints are numbered relative to the transcription start site of the distal promoter. The enhancers (boxes), promoters (D and P) are indicated. The *D*−5000 gene includes sequences from −5,000 to +2,510 and is expressed at wild-type levels in all developmental stages (V.C. and T.M. manuscript in preparation). The *ALE*/*P*−386 gene contains the ALE fused directly to −386 of the proximal promoter, deleting the AAE and the distal promoter (−386 of the proximal promoter equals +328 of the distal promoter). *ALE*/*P*−386 transformants produce proximal transcripts at levels ~3-fold (1 line), 5- to 10-fold (2 lines), 15- to 20-fold (2 lines), and ~30-fold (1 line) higher than wild-type controls. The internally deleted genes, *D*−660/−128, *D*−127/*D*−63, *D*−66/+53 and *D*+53/+328, remove the ALE (sequences between −660 and −128), sequences just upstream of the distal promoter (between −127 and −63), the distal promoter's TATA box and initiation site (sequences between −66 and +53), and the sequences downstream of the distal promoter (between +53 and +328). In *D*−66/+53 transformants, the activity of the proximal promoter was three-fold (3 lines) or 5- to 10-fold (4 lines) higher than wild-type, although no proximal promoter activity was observed in 2 other lines. *D*−660 contains sequences from −660 to +2,510 (V.C. and T.M. manuscript in preparation). 5′3′*AAE* contains one copy of the AAE in its normal location, and a second copy (−660 to −128) at the 3′ end of the gene (+3,100). 5′*P*3′*DP* contains a duplicate proximal promoter plus *Adh* gene body (+328 to +2,510) linked to a downstream *Adh* gene (−660 to +3,100). *P*−386 contains sequences from +328 (−386 of the proximal promoter) to +2,510 of the distal promoter.
Methods. Standard cloning procedures were used[15]. *Adh* constructs were cloned into the transformation vector Carnegie 20 (ref. 16). Germline transformants were made by injecting the clone of interest and the transposase plasmid, pπ25.7 wc (ref. 17), into ry^{506} embryos[18–20]. Transformants were crossed into the Adh^{fn6}, *cn*; ry^{506} background and established as homozygous stocks[21].

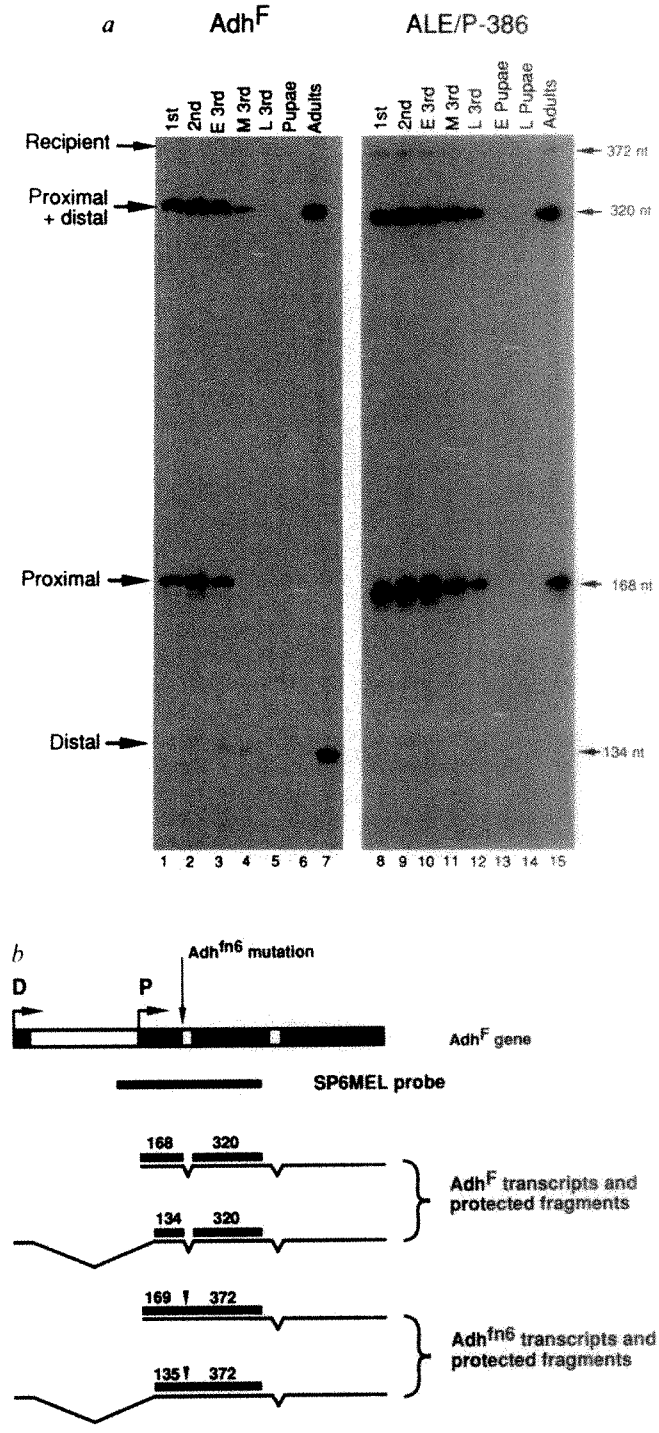


Fig. 3 The proximal promoter is aberrantly active in late third-instar larvae and in adults when the AAE and distal promoter are deleted. *a*, *Adh* transcript levels in *ALE/P−386* transformants were compared to wild-type controls at the indicated developmental stages by RNase mapping[22]. Relevant bands are marked at the side. *b*, Expected products from RNase mapping experiments using the *Adh*-specific probe SP6MEL (ref. 21). The transcribed portion of the Adh^F gene is shown. Black boxes indicate exons; white boxes, introns. The extent of the complementary single-stranded RNA probe, SP6MEL, is indicated by the shaded bar below. Bracketed drawings show RNase-resistant hybrids which form between the probe and transcripts of the transformed (Adh^F) and endogenous (Adh^{fn6}) *Adh* genes. Adh^F transcripts give rise to three protected fragments: a 320-nucleotide (nt) fragment, common to proximal and distal transcripts; a 168 nt fragment, specific to proximal transcripts; and a 134 nt fragment, specific to correctly spliced distal transcripts. Adh^{fn6} transcripts are not spliced properly due to a mutation near the 3′ splice site of the first small intron (marked by arrow)[23]. Distal Adh^{fn6} transcripts yield fragments of 135 and 372 nt; proximal transcripts, fragments of 169 and 372 nt.

Methods. Total nucleic acid was isolated from staged organisms as previously described[21] and hybridized to ^{32}P-labelled RNA probes complementary to *Adh* and α-tubulin transcripts. First, second, early third, mid third and late third-instar larvae were collected 30–42 h, 54–66 h, 66–78 h, 78–90 h and 90–102 h after egg laying, respectively. Early and late pupae were collected 24 and 72 h after the white pre-pupae stage. Adult males were collected 4 days after eclosion. RNA probes were made as described[24], except that bovine serum albumin was added to 10 μg ml^{-1}. RNase mapping experiments were as described[22], except hybridizations were at 37 °C, and the hybrids were digested for 30 min at 25 °C. The products were fractionated on 5% denaturing polyacrylamide gels and visualized by autoradiography.

of the position of the enhancers. The critical factor seems to be the position and activity of the distal promoter. The simplest interpretation of our data is that the distal promoter represses the proximal promoter by transcriptional interference.

Transcriptional interference was first shown to modulate promoter activity in *Escherichia coli*[3,4,8], and subsequently in vertebrates[5–7]. For example, induction of λ-prophage integrated upstream of the *E. coli gal* operon reduces transcription from the P_{gl} promoter[4]. Similarly, when the human α_1 globin gene is duplicated, transcription from the upstream gene inhibits transcription from the downstream gene[6]. Insertion of a transcription terminator between the two genes prevents this inhibitory effect[6]. Although the exact mechanism of transcriptional interference is not known, *in vitro* studies suggest that when RNA polymerase passes through a downstream promoter it can destabilize interactions between that promoter and specific transcription factors[7].

Here, we present evidence that transcriptional interference is important in the promoter switch of the *D. melanogaster Adh* gene. The proximal promoter is repressed in late third-instar larvae and adults apparently by readthrough transcription from the distal promoter. The proximal promoter seems to be sensitive to even low levels of transcription from the distal promoter (Fig. 4*a*, lanes 6–8), which may explain the rapidity of the switch from the proximal to distal promoter in third-instar larvae. Thus, the *Adh* promoter switch seems to be controlled largely or entirely by the stage-specific activation of the distal promoter which, in turn, may be regulated by the stage-specific activity

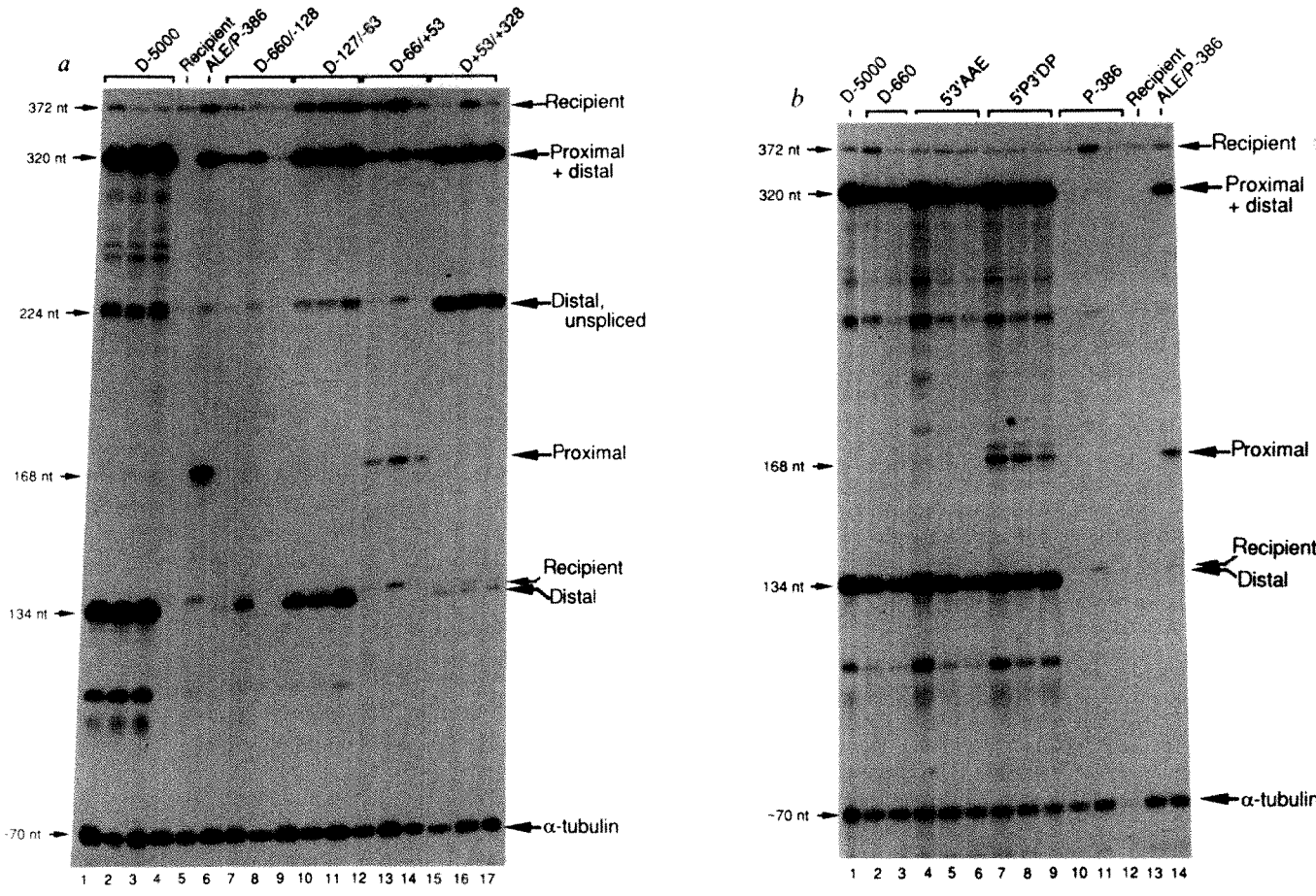


Fig. 4 The distal promoter is required to repress transcription from the proximal promoter in adults. Proximal and distal *Adh* transcripts from adult transformants were quantitated by RNase mapping. Each lane represents an independent transformed line of the indicated genotype. Relevant bands are marked at the side of the autoradiograms. *a*, In the absence of the AAE, the activity of the distal promoter was reduced 8- to 20-fold, but the proximal promoter was active at normal low levels ($D-660/-128$, lanes 6–8). Similarly, the activity of the distal promoter was reduced ~5-fold by the deletion of sequences just upstream of the distal TATA box, but the proximal promoter was unaffected ($D-127/-63$, lanes 9–11). The deletion of the sequence just downstream of the distal promoter did not affect the activity of either promoter ($D+53/+328$, lanes 15–17), although distal transcripts were not properly spliced due to the deletion of the 5′ splice site of the large intron[23]. In contrast, when the distal promoter's TATA box and transcription initiation site were deleted, the proximal promoter was expressed at levels 2- to 10-times higher than normal ($D-66/+53$, lanes 12–14). *b*, Proximal transcripts from *5′P3′DP* were detected at levels 10- to 15-fold higher than controls (compare lanes 7–9 to lanes 1–3). Note that both the upstream and downstream proximal promoters contribute to the pool of proximal transcripts. The downstream proximal promoter, however, is active at extremely low levels in controls (lanes 1–3). Therefore the vast majority of the proximal transcripts must initiate at the upstream proximal promoter.
Methods. Total nucleic acids from adult males were treated as described in Fig. 3 legend.

of the transcription factors that bind to it and the AAE (ref. 9 and V.C., D. Falb and T.M., unpublished observations).

Only one other example of a stage-specific promoter switch has been studied in detail. In the chicken β-globin locus the ε-globin gene is expressed in embryonic erythroid cells, and the closely linked β-globin gene is expressed in adult erythroid cells[10,11]. Recent studies have shown that the ε-globin gene is inappropriately expressed in adult cells if the β-globin gene promoter is deleted[12]. The promoter switch in both the *Adh* and globin systems seem to be regulated by the stage-specific activation of an adult promoter. In the globin locus, however, the ε-globin gene is repressed in adult cells by competing with the β-globin promoter for interactions with a single erythroid cell-specific enhancer[12]. In contrast, the distal *Adh* promoter represses the proximal promoter by transcriptional interference. Thus two distinct mechanisms appear to regulate promoter switching in these two systems.

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Mutation rates differ among regions of the mammalian genome

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In the traditional view of molecular evolution, the rate of point mutation is uniform over the genome of an organism and variation in the rate of nucleotide substitution among DNA regions reflects differential selective constraints[1,2]. Here we provide evidence for significant variation in mutation rate among regions in the mammalian genome. We show first that substitutions at silent (degenerate) sites in protein-coding genes in mammals seem to be effectively neutral (or nearly so) as they do not occur significantly less frequently than substitutions in pseudogenes. We then show that the rate of silent substitution varies among genes and is correlated with the base composition of genes and their flanking DNA. This implies that the variation in both silent substitution rate and base composition[3] can be attributed to systematic differences in the rate and pattern of mutation over regions of the genome. We propose that the differences arise because mutation patterns vary with the timing of replication of different chromosomal regions in the germline. This hypothesis can account for both the origin of isochores in mammalian genomes[4] and the observation[5] that silent nucleotide substitutions in different mammalian genes do not have the same molecular clock.

Early estimates of the substitution rates in pseudogenes and at silent sites in protein-coding sequences suggested that pseudogenes evolve more rapidly which implies that silent sites are under some selective constraint[6,7]. However, these studies used very limited data and rather simple methods of estimating rates. Here we use two approaches to compare the rate of substitution in functionless DNA (that is, in pseudogenes and intergenic regions) with that at silent sites in genes; the latter is measured in terms of the number of nucleotide substitutions per fourfold degenerate site (designated K_4). First, we compare the divergences in pseudogenes and in functional genes across the same species pair. If silent sites in functional genes are selectively constrained, then they should have accumulated fewer substitutions.

By far the most substantial data set available is for DNA sequences from humans and Old World monkeys (Table 1). The mean degree of divergence at silent sites is clearly not lower than that in pseudogenes. There are rate differences among genes, but for only one gene (β-globin) out of 13 is the silent rate significantly lower than the pseudogene rate, suggesting that the β-globin rate is an extreme random deviate. Nevertheless, perhaps a better method is to compare a recently generated pseudogene with its functional homologue from the same species, using the functional gene from a second species as a reference. This method can be applied to both processed and unprocessed pseudogenes, but is more accurate in the former case because processed pseudogenes can be assumed to become functionless as soon as they are formed. The three LDH-A processed pseudogenes in mouse[8] provide excellent data for this approach because they are reasonably large and because they were produced after the mouse-rat split, so the rat LDH-A gene can be used as a reference. The ratios of the substitution rates in the three LDH-A pseudogenes to the rate at silent sites in the functional gene are 1.1, 0.8 and 0.7. Again, there is little, if any, evidence for a significantly higher rate of substitution in pseudogenes than at silent sites. We therefore conclude that most silent (fourfold degenerate) sites are effectively neutral, and that their substitution rate is a good measure of the mutation rate, because under selective neutrality the substitution rate is expected to be equal to the mutation rate[1].

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Table 1 Rates of nucleotide substitution at silent sites and in functionless DNA for human versus Old World monkey sequences

Gene	$K_4 \pm$ s.e.m.	GC_4*	L_4†
α_1-Antitrypsin	0.077 ± 0.022	73.6	184
Apolipoprotein A-I	0.061 ± 0.025	88.4	126
Apolipoprotein E	0.062 ± 0.021	89.1	175
Chorionic gonadotrophin-β	0.065 ± 0.027	81.3	99
Erythropoietin	0.110 ± 0.035	71.7	110
β-Globin	0.039 ± 0.028	68.2	54
Insulin	0.130 ± 0.054	82.3	62
Metallothionein I	0.125 ± 0.113	85.5	21
Metallothionein II	0.096 ± 0.072	87.0	23
β-Myosin heavy chain	0.179 ± 0.056	86.5	97
Pepsinogen A	0.108 ± 0.026	73.7	194
Transforming growth factor-β	0.064 ± 0.019	83.5	203
Triose phosphate isomerase	0.112 ± 0.033	59.8	128
Total for silent sites	0.089 ± 0.009	78.7	1,474
η-Globin pseudogene locus	0.074 ± 0.006	42.9	2,071
η-δ Globin intergenic DNA	0.076 ± 0.006	38.7	2,771
Total for functionless DNA	0.075 ± 0.004	40.5	4,842

K_4 (the corrected number of substitutions per fourfold degenerate site) was calculated as described in Fig. 1. References to the original DNA sequence data are available on request.
* G+C content at fourfold degenerate sites (%).
† Number of fourfold degenerate sites compared.

Next we focus on the variation among genes in the substitution rate at silent sites (K_4) and show that it is correlated with their G+C content (GC_4). We compare DNA sequences between mouse and rat, first concentrating on the larger genes (those with over 175 silent sites) (Fig. 1*a*). For the 17 genes with $GC_4 \geq 50\%$, K_4 clearly decreases as GC_4 increases (correlation coefficient $r = -0.73$, $P < 10^{-4}$); the highest K_4 is nearly twice the lowest value. On the other hand, there is a significant positive correlation between substitution rate and GC_4 in the A+T-rich genes, though the number of points is small ($r = +0.82$; $P < 0.05$). This latter observation suggests that the low substitution rates in genes with a high G+C content cannot be simply explained by assuming that G and C nucleotides mutate less frequently than A and T nucleotides. On reflection, it is difficult to think of any other reasonable relationship between K_4 and GC_4, although this has not previously been reported. In fact, others have suggested either no systematic variation (in silent substitution rate) among genes[9], or a simple linear relationship between substitution rate and G+C content[10,11]. When smaller genes are included (making a total of 88 genes), the same peaked relationship as that shown in Fig. 1*a* is seen, but the correlation coefficients are decreased because of the stochastic effects (particularly on K_4) of short genes. The above comparison requires no knowledge of the date of speciation, the data set used is large, and the K_4 values are fairly large but far from saturation. The mouse-rat comparison is preferable to a human-rodent comparison because of the shorter timescale involved; some human genes differ considerably in base composition[12] or even in chromosomal map position[13] from their rodent homologues.

The variance of GC_4 in the 23 large rodent genes (Fig. 1*a*) is more than ten times that expected by chance (under a binomial distribution with mean 58.6%), suggesting that the factors determining G+C content are not uniform for all genes. Of course, one might argue that the GC_4 value in any gene is at a particular optimum and that the observed relationship between K_4 and GC_4 is a consequence of selection maintaining that GC_4 value in the face of a mutation rate that is uniform across the genome. But this would require strong selective constraints, whereas we have shown here that silent sites seem to be effectively neutral. Furthermore, the direction of selection on G+C-rich genes

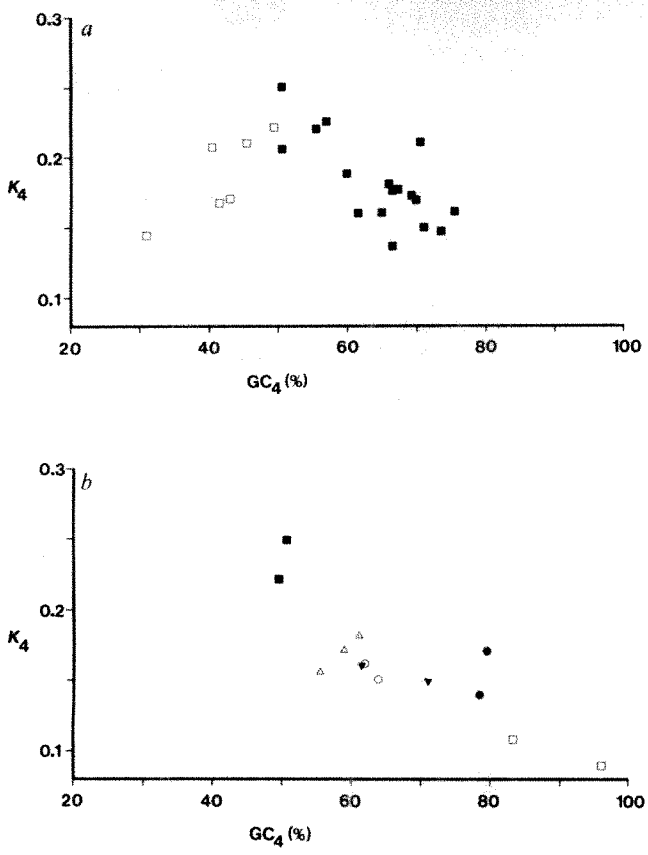


Fig. 1 *a*, Plot of silent substitution rate (K_4) versus G+C content at silent sites (GC_4) for 23 genes compared between mouse and rat. Only large genes (with ≥175 fourfold degenerate sites) are compared. ■, 17 genes for which $GC_4 \geq 50\%$ (encoding alcohol dehydrogenase-1, α-fetoprotein, CD4 antigen, c-*mos* and c-*myc* oncogenes, creatine kinase M, cytochromes $P_1$450 and $P_3$450, β-glucuronidase, malic enzyme, mitochondrial aspartate aminotransferase and malate dehydrogenase, neural cell adhesion molecule NCAM-140, neurofilament M, oestrogen receptor, protein disulphide isomerase and renin); □, 6 genes with $GC_4 < 50\%$ (encoding albumin, α_2-amylase, glucocorticoid receptor, leukocyte common antigen (Ly-5, T200), ornithine decarboxylase and UDP-glucuronosyltransferase). Full references for the sequences are available from the authors on request; all are taken from GenBank (release 50) or the recent literature. K_4 was calculated by the method of Tajima and Nei[30], which requires no assumptions about the base composition of the sequences compared, other than that they be at equilibrium. Any codons at which mouse and rat differed by more than one nucleotide substitution were ignored. This avoided (1) the need to apply weights to the different mutational pathways possible between multiply-substituted codons, and (2) any regions within a gene in which the protein sequences of the two species are considerably different and might not share a common origin (for example as a result of insertions, deletions or alternative splicing). If, as we assume, the sequences being compared have diverged only through a process of random point mutation, ignoring these codons should not alter the synonymous substitution rate calculated. *b*, Relationship between K_4 and GC_4 for several groups of physically linked genes: □, metallothioneins I and II (5 kb apart); ■, albumin and α-fetoprotein (14 kb); ▼, cytochromes $P_1$450 and $P_3$450 (<25 kb); ●, γ_A and γ_D crystallins (35 kb); ○, Ly-2 and Ly-3 antigens (36 kb); △, immunoglobulin heavy chains C_δ $C_{\gamma 3}$ and C_ε (50 kb and 90 kb).

would have to be opposite to that on A+T-rich genes. Thus our finding that the substitution rate and the base composition of silent sites vary together in a systematic way is most simply explained by supposing that the pattern of mutation is different for different genes. Most germline mutations are thought to arise from misincorporation errors made by the DNA replication apparatus[14,15]. It has been demonstrated that different genes replicate at different stages of the cell cycle in differentiated cells[16], and this is presumably also true in the germline. The number and type of replication errors are likely to vary during the cell cycle if the chemical environment in the nucleus changes. In fact, the abundances (both relative and absolute) of free dNTPs in the nucleus change with time[17], as do the activities of the DNA polymerase enzymes and their accessory proteins[18]. We have examined a theoretical model (to be detailed elsewhere) of the relationship between the mutation rate and the G+C content of both the nucleotide precursor (dNTP) pools and the template DNA. This model is based on a simple kinetic description of DNA replication[19], as applied to the replication of long double-stranded DNA molecules. It can explain the occurrence of the maximum mutation rate at an intermediate value of GC_4, provided that GC_4 is positively correlated with the proportion of dGTP plus dCTP in the dNTP pools. This assumption seems plausible as we are considering only effectively neutral sites in DNA. There is also some experimental evidence to support this: as S-phase progresses, the G+C content of both the dNTP pools and the replicating DNA decreases[16,17,20]. As K_4 is a consequence of the mutation rate, under this model the relationship seen in Fig. 1*a* can be generated in the absence of selection.

Replication origins in mammalian DNA are located 50–300 kilobases (kb) apart, and clusters of 12–100 adjacent origins tend to replicate simultaneously[16]. Thus, regions of DNA of 1,000 kb or more could form temporal units of replication, and all DNA within a unit would be expected to show the same pattern and rate of mutation. Thus, under our model, genes which are physically close to each other in the genome should have similar values of K_4 and GC_4. This is indeed the case for those genes for which data are available (Fig. 1*b*): several pairs of genes which are the results of ancient tandem duplications show paired results for K_4 and GC_4, as do genes of the IgH constant-region locus. These IgH genes span 140 kb on mouse chromosome 12, and are known to be part of a single replicon[21].

These replication units seem to correspond to the 'isochores' (large blocks of DNA with homogeneous base compositions) found experimentally in mammalian nuclear DNA[4], and probably also to high-resolution Giemsa chromosomal bands[16]. We therefore propose that isochores arise as a result of the synchronous replication of megabase stretches of DNA under varying dNTP pool conditions. Although our model based on variation in the dNTP precursor pools can provide a simple explanation for the observed variation of mutation rates and patterns around the genome, it is of course not the only possible explanation. For example, Filipski[22] has proposed that isochores are formed as a consequence of the repair of DNA in different types of chromatin by different DNA polymerase enzymes, and there is now some experimental evidence for between-gene differences in efficiency of DNA repair[23]. Our observations could also be explained if DNA is replicated by several distinct DNA polymerase holoenzymes with different error propensities. But recent data indicate that there is only one replicative polymerase complex for mammalian nuclear DNA[18,24].

We conclude that much of the intragenomic variation in silent substitution rate and base composition in mammals results from variation in the process of mutation, rather than from natural selection[25,26]. The implication that silent sites in mammalian genes are effectively neutral contrasts with prokaryotic genes, in which differences in translational efficiency lead to selective differences between codons[27] and thus to lower silent substitution rates in genes that are highly expressed[2]. Our assertion is supported by several arguments, however. First, the base composition of silent sites in mammalian genes is strongly correlated with that of introns and flanking sequences of the same gene[3],

suggesting that it is determined largely by local mutation pressures. In addition, it has recently been suggested on the basis of analysis of dinucleotide frequencies that codon usage in human genes is determined by context-dependent mutational patterns[28]. Second, any suggestion of tissue-specific or expression level-related patterns in mammalian codon usage must be set against the observation that the human α- and β-globin genes have very different codon usage. Third, as the effective population sizes of mammalian species are quite small[29], it would require large selective differences for selection to be effective, but it is unlikely that mammals have sufficient reproductive excess to allow such selection at a very large number of codons. In summary, we propose that, in the mammalian genome, variation in the silent substitution rate and variation in base composition are two facets of the same phenomenon.

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Nonmutagenic carcinogens induce intrachromosomal recombination in yeast

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To identify environmental carcinogens there is a need for inexpensive and reliable short-term tests[1], but certain human or animal carcinogens are persistently undetectable as mutagents with the Ames assay[2–5] or with other short-term tests currently in use[6,7]. Thus there is a need for short-term tests which detect carcinogens missed by the Ames assay[1]. Because of the association of carcinogenesis with genome rearrangement[8–12], a system screening for intrachromosomal recombination resulting in genome rearrangement has been constructed in *Saccharomyces cerevisiae*[13]. Evaluation of this system shows inducibility by a variety of carcinogens[3–7] not detectable by the Ames assay or various other short-term tests. In the light of these results it is tempting to speculate that 'nongenotoxic carcinogens' are in fact genotoxic but, in the past, the tools to measure the genetic alterations they induce have been inappropriate.

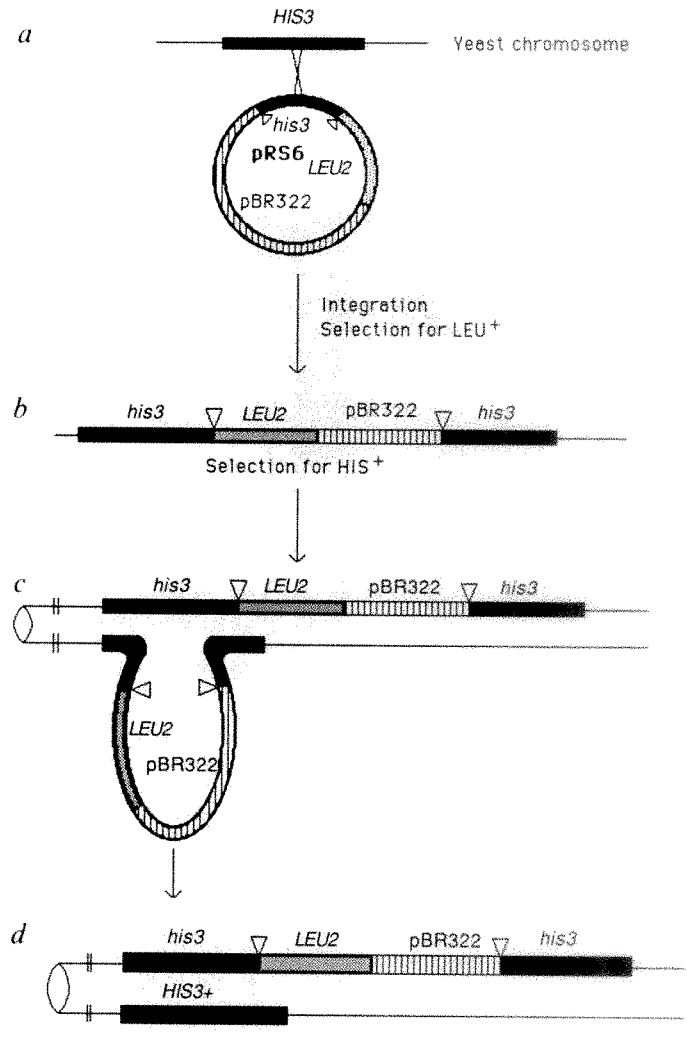


Fig. 1 Plasmid pRS6 which contains an internal fragment of the *HIS3* gene was cut within the internal *his3* fragment and integrated into the geome at the *HIS3* locus *a*. This creates a duplication of the *his3* gene in which one allele is deleted for its 3′ end and the other for its 5′ end *b*, The two alleles share about 400 base pairs of homology and thus can recombine with each other to revert to the $HIS3^+$ allele. As shown previously $HIS3^+$ recombinants do not arise by plasmid excision or by unequal sister chromatid exchange[13]. In these studies the frequency of plasmid excision was determined by putting a yeast origin of replication sequence onto the integrating plasmid. Excised plasmids could be recovered but were 100-fold less frequent than the frequency of HIS^+ formation which suggests that plasmid excision does not occur in the majority of recombinants. Reciprocal products expected to result from sister chromatid exchange were analysed by Southern blotting. The pattern characteristic for sister chromatid exchange was not found in any of 25 events examined. Thus, as a likely alternative mechanism conversion between sister chromatids (*c*) is suggested which result in deletion of the integrated plasmid on one chromatid (*d*). After segregation of the two chromatids the HIS^+ recombinant should show a HIS^+ leu^- phenotype which is found in 99% of all HIS^+ recombinants[13]. Thus, it is proposed, as a possible model, that a double strand break initiates the recombination event which is extended by an exonuclease to a gap. This gap is repaired by gene conversion from the sister chromatid as donor[13].

Table 1 Carcinogens which are detectable with the Ames assay

Carcinogen	Response in DEL (concentration range in μg ml^{-1})	Response in ICR (concentration range in μg ml^{-1})	Concentration in μg ml^{-1}	HIS$^+$ per 10^4 cells (colonies counted)	ADE$^+$ per 10^5 cells (colonies counted)
MMS[18]	Positive (1.3–260)	Positive (1.3–260)	0	0.97 (87)	0.45 (20)
			260	129 (137)	62 (654)
EMS[19]	Positive (234–2,340)	Positive (234–2,340)	0	0.97 (87)	0.45 (20)
			2,340	25.3 (1,428)	28.9 (155)
NQO[20]	Positive (0.1–2)	Positive (0.1–2)	0	0.97 (87)	0.45 (20)
			2	55.6 (562)	106 (252)
Nitrogen mustard[3]	Positive (0.1–100)	Positive (0.1–100)	0	0.79 (73)	1.3 (120)
			100	176 (84)	460 (22)
Epichlorohydrine[18]	positive (100–400)	Positive (100–400)	0	0.68 (76)	0.58 (65)
			400	48 (2,976)	50 (620)
Aflatoxin B_1[18]	Positive (2.5–50)	Positive (2.5–50)	0	0.68 (76)	0.58 (65)
			50	12.5 (277)	3.15 (70)
1-2 Dibromoethane[22]	Positive (22–4,400)	Positive (22–4,400)	0	1.2 (127)	3.3 (328)
			4,400	3.8 (31)	12.2 (10)
Dimethylhydrazine[18,23]	Positive (10–5,000)	Positive (10–5,000)	0	0.78 (75)	2.5 (245)
			5,000	3.9 (131)	4.7 (16)

The influence of MMS exposure (vol/vol) on the frequency of deletions (DEL) and interchromosomal recombination (ICR) was determined The diploid strain RS112 (MATa/α *ura3-52/ura3-52 leu2-3,112/leu2-Δ98 trp5-27/TRP5 arg4-3/ARG4 ade2-40/ade2-101 ilv1-92/ILV1 HIS3*::pRS6/*his3-Δ200 LYS2/lys2-801*) was used and contains the DEL system on one homologue (*HIS3*::pRS6) and a deletion of the entire open reading frame of *HIS3* on the other homolog (*his3-Δ200*)[27]. Single colonies were picked from YPAD medium[28] and were inoculated into 5–25 ml of synthetic complete medium lacking leucine (SC-LEU)[28] and grown for 24 hours at 30 °C under constant shaking. Cells were counted and the cell density was adjusted to 2×10^6 cells ml^{-1} in fresh SC-LEU medium. The medium containing the cells was distributed in aliquots of 5 ml each into disposable 15-ml tubes. The agent to be tested was added, the tubes sealed, and the cells were incubated for 17 hours at 30 °C under constant shaking. Cells were pelleted in a clinical table-top centrifuge. After washing the cells twice with sterile distilled water or a 10% solution of sodium thiosulphate to inactivate the agent, cells were resuspended in 0.5–1 ml of sterile distilled water, transferred to a glass tube and sonicated to disperse any clumps. Cells were counted and appropriate numbers were plated onto SC medium to determine the number of survivals, onto SC-HIS medium to score for DEL events and onto SC-ADE medium to determine the number of ICR events. The cells did not contain any clumps or tetrads. Colonies were counted after two to three days of incubation at 30 °C. Numbers of recombinants were calculated per numbers of survivors Shown is the concentration of the agent, the number of generations obtained after 17 hours of incubation, the survival and DEL as well as ICR frequencies. Data derived from less than five colonies were not included. The same control was used for MMS, EMS and NQO as well as for epichlorohydrin and aflatoxin exposure respectively. A minimum increase of twofold over the spontaneous frequency in a dose dependent manner has been regarded as evidence for inducibility. Each experiment was repeated at least three times and the results obtained were highly reproducible two plates were used for each system and each concentration. The same qualitative result was obtained with other diploid as well as haploid strains containing the DEL system. MMS and EMS were purchased from VWR Scientific and purified by vacuum distillation at 10–15 mm Hg pressure before use. NQO, nitrogen mustard, dibromoethane (ethylene dibromide), epichlorohydrine, aflatoxin and dimethylhydrazine were obtained from Sigma.

There is a large body of data establishing that animal and human carcinogens without apparent genotoxic activity exist[6]. This is the basis for the concern that there may be a considerable number of nonmutagenic carcinogens in our environment that are not detected by the conventional short term tests. As models for carcinogenesis of 'nongenotoxic carcinogens' are largely missing, a significant regulatory dilemma in their assessment of risk to humans exists[14].

There is evidence that substantial genome rearrangements are associated with cancer[8–12]. Furthermore deletions in recessive oncogenes have been implicated in carcinogenesis such as retinoblastoma[15,16]. Because of the association of genome rearrangement with cancer, a system selecting for intrachromosomal recombination which results in genome rearrangement has been constructed in the yeast *S. cerevisiae*[13] (Fig. 1). A plasmid with an internal fragment of the *HIS3* gene has been integrated at the *HIS3* locus yielding an integrative disruption of the *HIS3* gene. This resulted in two copies of the *HIS3* gene each having one terminal deletion. The most likely mechanism for reversion seems to be sister chromatid conversion between the two *his3*$^-$ alleles[13]. Because this recombination event deletes the integrated plasmid (Fig. 1) it is termed a deletion (DEL) event.

The DEL system is under different genetic control than the previously described recombination systems interchromosomal recombination (ICR) and meiotic recombination. It has been shown that mutations in the DNA repair gene *RAD1* affect the DEL recombination but have no effect on ICR or meiotic recombination[17]. This suggests that the mechanism for DEL recombination differs from that for ICR as well as meiotic recombination.

To determine whether DEL is inducible by carcinogens the diploid strain RS112 was used in which all recombination events have to occur between the two *his3*$^-$ alleles on one chromosome because the entire *HIS3* gene is deleted on the homologous chromosome. RS112 is also heteroallelic for *ade2* to measure interchromosomal recombination (ICR) between homologous chromosomes and the two recombination systems can be compared in their performance to detect carcinogens.

As mutagenic carcinogens methyl methanesulphonate (MMS)[3,18], ethyl methanesulphonate (EMS)[3,19], 4-nitroquinoline-*N*-oxide (NQO)[3,7,20], nitrogen mustard[3], epichlorohydrine[5,7,18] and aflatoxin B_1[3,18] were used (see Table 1 for procedure) and gave strong inductions with the DEL system (HIS$^+$ 20–220-fold, Table 1) as well as with the ICR system (ADE$^+$ also see ref. 21). Ethylene dibromide[22] is weakly positive with the Ames assay[3], and dimethylhydrazine[18,23] which has been reported negative[3] or weakly positive[5] with the Ames assay, both induce DEL recombination but ICR to a lesser extent (Table 1

The carcinogens formaldehyde, safrole, ethionine, urethan, auramine, methylene chloride, carbon tetrachloride, cadmium chloride and sulfate, aniline, aminotriazole, acetamide, thioacetamide, thiourea, dichlorodiphenyldichloroethylene (DDE) and ethylenethiourea are negative with all five strains currently used in the Ames assay[3,5,22], yet they have been shown to be animal or suspected human carcinogens (see Table 2 for references). All of these carcinogens induce the DEL system (2–13-fold; see Table 2).

In an international collaborative study with coded chemicals evaluating 30 different short term assays in more than 50 different laboratories[7] the carcinogens auramine, safrole, urethan

Table 2 Agents which are not detectable as 'carcinogens' in the Ames assay

Agent*	Response in DEL (concentration range in μg ml^{-1})	Response in ICR (concentration range in μg ml^{-1})	Concentrations (μg ml^{-1})	HIS$^+$ per 10^4 cells (colonies counted)	ADE$^+$ per 10^5 cells (colonies counted)
Formaldehyde[23,29]	Positive (15-70)	Positive (15-50)	0	2.3 (212)	0.49 (45)
			50	29.5 (109)	5.4 (6)
Safrole[7,18,23]	Positive (2,000-4,000)	Negative (up to 2,000)	0	1.1 (129)	0.50 (55)
			2,000	2.4 (117)	0.82 (40)
Ethionine[7,18,30]	Positive (1,000-20,000)	Negative (up to 20,000)	0	1.05 (108)	0.66 (68)
			20,000	5.4 (250)	0.80 (47)
Urethane[7,18,23]	Positive (20,000-40,000)	Negative (up to 20,000)	0	0.97 (99)	0.45 (47)
			20,000	4.8 (478)	0.40 (20)
Auramine[18,31]	Positive (1,200-1,500)	Negative (up to 1,200)	0	1.2 (127)	3.3 (328)
			1,200	2.64 (78)	1.7 (5)
Methylene chloride[22,29,31]	Positive (10,000-12,000)	Negative (up to 12,000)	0	0.95 (119)	1.1 (693)
			12,000	3.6 (109)	0.98 (5)
Carbon tetrachloride[18,23,29]	Positive (4,000-8,000)	Negative (up to 4,000)	0	1.05 (108)	0.66 (68)
			4,000	2.0 (99)	0.61 (6)
Cadmium chloride[18,31]	Positive (100-700)	Negative (up to 400)	0	0.94 (95)	0.49 (55)
			400	3.5 (145)	0.30 (8)
Cadmium sulphate[18,31]	Positive (100-700)	Negative (up to 400)	0	0.94 (95)	0.49 (55)
			200	2.6 (516)	0.2 (5)
Aniline[22]	Positive (4,000-7,000)	Positive (at 7,000)	0	1.18 (134)	1.34 (153)
			7,000	7.9 (321)	4.9 (20)
3-Aminotriazole[18]	Positive (40,000-60,000)	Negative (up to 60,000)	0	1.13 (91)	1.08 (86)
			60,000	3.1 (798)	0.7 (19)
Acetamide[18]	Positive (20,000-40,000)	Negative (up to 40,000)	0	1.13 (91)	1.08 (86)
			40,000	5.04 (782)	0.45 (7)
Thioacetamide[18]	Positive (10,000-20,000)	Negative (up to 20,000)	0	1.13 (91)	1.08 (86)
			20,000	6.28 (1,432)	1.14 (26)
Thiourea[18]	Positive (10,000-30,000)	Positive (at 30,000)	0	1.13 (91)	1.08 (86)
			30,000	25.3 (76)	20 (6)
DDE[22]	Positive (100-5,000)	Positive (at 5,000)	0	1.99 (112)	0.57 (32)
			5,000	14.1 (97)	4.3 (5)
Ethylenethiourea[7,18]	Positive (20,000-40,000)	Positive (at 40,000)	0	0.97 (85)	0.56 (49)
			40,000	3.1 (576)	1.5 (32)
Methionine	Negative (up to 40,000)	Negative (up to 40,000)	0	0.95 (119)	1.1 (693)
			40,000	0.64 (66)	0.69 (358)

The experiment was carried out as described in Table 1. The controls for the agents ethionine and carbon tetrachloride, for cadmium chloride and sulphate, for 3-aminotriazole and acetamide, and for thioacetamide and thiourea were the same. Formaldehyde, methylene chloride (dichloromethane) and carbon tetrachloride were obtained from J. T. Baker Chemical Company. Safrole, ethionine, auramine, cadmium chloride, cadmium sulfate, aniline, 3-aminotriazole, acetamide, thioacetamide, thiourea and methionine were from Sigma. Urethane, DDE (2.2-bis[4chloro-phenyl]-1,1dichloroethylene) and ethylenethiourea (2-Imidazolidinethione) were from Aldrich.

* References are given for the carcinogenicity of carcinogenic agents.

ethionine, ethylenethiourea, and 3-aminotriazole gave very poor inducibilities with all short term tests.[7]. In the collaborative study as many short term tests were positive with the amino acid methionine as were positive with the carcinogen ethionine and only very few tests could differentiate between the two compounds[7]. After incubation in up to 40 mg ml^{-1} of methionine no increase of the frequency of DEL was detectable (Table 2), whereas ethionine gave already a twofold increase at a concentration of 1 mg ml^{-1} (Table 2). Thus the DEL assay can readily differentiate between methionine and ethionine.

Formaldehyde, safrole, ethionine, urethane, 3-aminotriazole and ethylenethiourea have been shown to induce chromosome mal-segregation in fungi which may be caused by a nongenotoxic mechanism[24]. Chromosome mal-segregation associated with DEL events would show a HIS$^+$ LEU$^+$ phenotype[13]. No evidence for an increase of the frequency of HIS$^+$ LEU$^+$ colonies was found. Therefore chromosome mal-segregation does not contribute significantly to the carcinogen induced frequency of HIS$^+$ formation.

In comparing the performance of the two systems, DEL and ICR, it will be noticed that the mutagenic carcinogens induce both systems well (Table 1). The nonmutagenic carcinogens on the other hand induce the DEL system much better than the ICR system (Table 2), and the carcinogens safrole, ethionine, urethane, auramine, methylene chloride, carbon tetrachloride, cadmium sulfate and chloride, aminotriazole and acetamide would not have been detectable with the ICR system. The deletion of the integrated plasmid (see Fig. 1), which is inducible by the 'nongenotoxic carcinogens' used, indicates a genotoxic activity of these carcinogens.

In some respects DEL induction and carcinogenesis may show similar characteristics. It may be one feature of nonmutagenic carcinogens that long term continuous exposure often at toxicologically significant doses is required to induce tumours[6] which also seems to be true for DEL induction (Table 2). It also seems that nonmutagenic carcinogens may have a threshold in their dose response curve which may be missing with many mutagenic carcinogens[25]. Similarly safrole (Fig. 2*c*), carbon tetrachloride, aniline, 3-aminotriazole, acetamide, ethylene thiourea, urethane (Fig. 2*d*) and thioacetamide all show thresholds below which no response can be detected. To the contrary the mutagenic carcinogens MMS (Fig. 2*a*), EMS, NQO, nitrogen mustard (Fig. 2*b*), dibromoethane, and aflatoxin increase the frequency of DEL recombination with increasing dose over a range of at least 10- to 1,000-fold (Table 1). Another similarity between carcinogenesis and DEL recombination was revealed when cells from Bloom's syndrome patients, a disease associated with a greatly increased incidence of cancer, were shown to contain an altered, heat-labile form of DNA ligase I[26]. Similarly DEL recombination is induced about 100-fold in a yeast *cdc9* mutant temperature sensitive for DNA ligase[17].

The result of this study may give an indication about molecular events involved in carcinogenesis. DEL events may give rise to recombination between repeated elements yielding genome

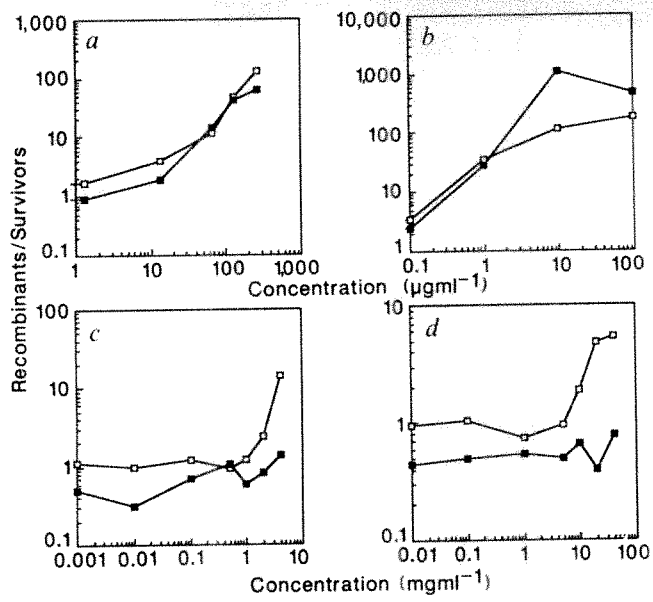


Fig. 2 Examples of dose-response curves for DEL (□, events per 10^4 survivors) as well as ICR (■, events per 10^5 survivors) recombination with two carcinogens which are detectable with the Ames assay, MMS (*a*) and nitrogen mustard (*b*) and two carcinogens which are not detectable with the Ames assay, safrole (*c*) and urethane (*d*). MMS and nitrogen mustard induce DEL as well as ICR in a dose dependent manner over a broad range of concentrations (1,000×) whereas safrole and urethane induce only DEL recombination and show a threshold, below which no induction can be detected.

rearrangements and bringing proto-oncogenes[8] into proximity with promoter or enhancer sequences. Naturally sister chromatid conversion may be important for the repair of damage induced in the S or G_2 phase and the nonmutagenic carcinogens may damage DNA specifically and only at this stage.

The DEL assay may exhibit more sensitivity to detect certain kinds of DNA damaging agents which are missed with the tests currently used. A large number of chemicals have to be tested to evaluate the DEL assay for its potential use in predictive carcinogenesis. This assay shows high reproducibility, is inexpensive, produces results within three days and requires only basic equipment and basic training in microbiology. If after further evaluation, results obtained with the DEL assay show high correlation with the carcinogenicity of the test agents, the DEL assay will be useful as a short term test for predictive carcinogenesis.

I am grateful to Professor U. Wintersberger at the Department for Tumour Biology, University of Vienna, under whose influence I developed the idea of constructing the DEL system. Dr R. D. Mehta of Prairie Biological Research Inc. is especially thanked for his help with the present work. Part of this study was carried out in the laboratory of Dr S. Prakash and was supported by Public Health Service grants from the NCI to S. Prakash. Drs R. D. Gietz, R. D. Mehta and Phil Hastings are thanked for many useful comments and for critical reading of the manuscript.

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Absence of significant RNA-dependent DNA polymerase activity in lymphocytes from patients with Kawasaki syndrome

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Kawasaki syndrome, an acute febrile multisystem illness of youn children, is a panvasculitis with prominent rheumatic features[1] Arthritis and pancarditis are frequent during the acute stage coronary artery aneurysms occur in 20% of cases and the diseas is now the leading cause of acquired heart disease in childhood A microbial aetiology is suggested by the acute febrile self-limite character of the disease, the regular occurrence of epidemic out breaks at intervals of 2–3 years, and the virtual restriction t young children, consistent with the early acquisition of immunit Reports of elevated DNA polymerase activity (assumed to b RNA-dependent reverse transcriptase) in cultured lymphocyte from patients with acute Kawasaki syndrome suggest that a retr virus might be the causative agent[2,3]. We have measured supe natant DNA polymerase activity in lymphocyte cultures from 4 Hawaiian patients in acute and convalescent stages of Kawasa syndrome and have been unable to demonstrate significant rever transcriptase activity or other evidence of involvement of a retr virus in the aetiology of the disease.

We established multiple cultures (two to four per patien from peripheral blood lymphocytes of 36 patients with acu Kawasaki syndrome (KS) at intervals from days two to 40 more of disease. An additional 13 patients were studied at or year or longer from onset of illness. Twenty-one KS patien

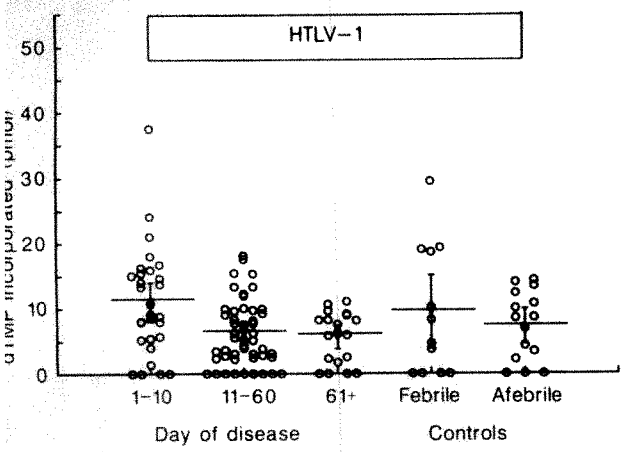


Fig. 1 Peak polymerase activity in the particulate fraction of supernatant fluids from cultures of lymphocytes collected from controls and KS patients at different stages of disease. Lymphocytes were isolated from peripheral blood of patients diagnosed as having KS by Dr Marian Melish using standard clinical criteria[6]. The mean age of the patients studied in the acute phase of disease was 29 months (range 4 to 94 months); that of control patients with febrile illness from infection with known or suspected viral or bacterial agents was 62 months (range 6 to 168 months); that of afebrile controls was 36 months (range 4 to 132 months).

Methods. Lymphocytes collected from heparinized blood by Ficoll-Hypaque sedimentation were cultured at a concentration of $1-2\times10^6$ per ml in Roswell Park Memorial Institute RPMI 1640 medium supplemented with 10% fetal bovine serum, glutamine, 2 mM final concentration, sodium bicarbonate sufficient to adjust final pH to 7.2, 100 units ml^{-1} each of penicillin and streptomycin, and phytohaemagglutinin (10 $\mu g\ ml^{-1}$). After 2–3 days incubation at 37 °C in 5% CO_2, the culture medium was decanted and replaced with complete medium supplemented with 0.5 $\mu g\ m^{-1}$ interleukin-2, 0.1% anti-human interferon-α, and 1.0 $\mu g\ ml^{-1}$ Polybrene. After 5–8 days in culture, 3×10^6 Hut-78 or Molt-4 cells or activated fresh human lymphocytes were added to each 5.0 ml culture. Culture supernatant fluids were collected at 3–5 day intervals for 21–28 days and assayed for reverse transcriptase activity. On day 21, idoxuridine 100 $\mu g\ ml^{-1}$ was added to selected cultures for 24–48 h, and then replaced with medium without idoxuridine. Supernates were collected 4–5 days later. Reverse transcriptase assays were performed[7]. Briefly, culture supernates were centrifuged in a Sorval RC-2B angle-head rotor at 2,000 r.p.m. for 10 min at 4 °C to remove intact cells and the supernatant fluid recentrifuged at 12,000 r.p.m. for 20 min to sediment cellular debris. The supernatant material was subsequently centrifuged at 25,000 r.p.m. for 90 min in an SW 28 rotor in a Beckman ultracentrifuge. In some cases, frozen (−70 °C) pre-clarified culture supernatant fluids collected on days 3–9, 10–13, or 17–28 respectively were pooled before untracentrifugation. The pellets were resuspended in 500 µl TNE buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, *p*H 8.0), freeze-thawed twice, and a 10-µl aliquot was added to 10 µl reaction mixture containing 83 $\mu g\ ml^{-1}$ dATP, 50 mM Tris-HCl, *p*H 8.0, 5 mM dithiothreitol, 5 mM $MgCl_2$, 50 mM KCl, 0.05% Triton X-100, 0.3 mM glutathione, 0.5 mM EGTA, 50 $\mu g\ ml^{-1}$ rADT, and 25 $\mu g\ ml^{-1}$ bovine serum albumin. After 5 min on ice, 7.5 µmol [^{3}H]dTTP were added and the mixture incubated at 37 °C for 60 min with repeated shaking. Then 10 µl RNA (10 mg ml^{-1}) was added and the reaction stopped by the addition of ice-cold 0.01M sodium pyrophosphate and 10% trichloroacetic acid (TCA). The precipitate was collected on 24 mm Whatman GFA glass filters pre-soaked in 5% TCA. The filters were rinsed with chilled 0.01M sodium pyrophosphate in 1M HCl, dried, placed in scintillation cocktail (Acquasol-II, NEN), and counted in a Beckman beta scintillation spectrometer. Negative control (uninfected Hut-78 or Molt-4 cell) and positive control (HIV-1 and/or HTLV-I) culture supernates were included in all tests. Mean plus/minus 95% confidence intervals are indicated. The open bar across the top of the figure indicates 95% confidence intervals about the mean for cells infected with HTLV-I

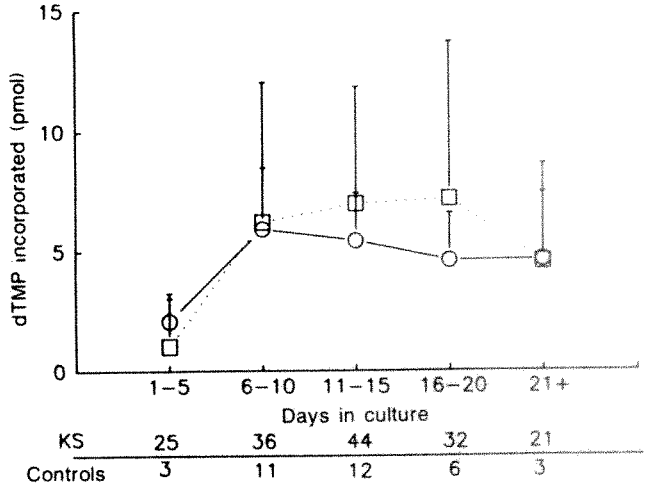


Fig. 2 Particulate polymerase activity in supernatant fluids of lymphocyte cultures. (○), KS patients in acute (febrile) phase; (□), febrile controls. The number of cultures tested at each time interval is shown on the horizontal line. Vertical brackets indicate 95% confidence intervals. Number of patients and control specimens tested at each time point is indicated below the graph.

were tested for serum antibodies to known human retroviruses—human immunodeficiency viruses types 1 and 2 (HIV-1, HIV-2) and human T-cell leukaemia viruses types I and II (HTLV-I, HTLV-II)—using the radioimmunoprecipitation assays previously described[4]. All patients except one were seronegative: one child with Japanese ancestry was seropositive for HTLV-I. DNA polymerase activity in supernatant fluid of lymphocyte culture was always low, with values significantly less than those of cell lines infected with HIV-1, HIV-2, and HTLV-I. The peak supernatant reverse transcriptase activity at every stage of the illness from the acute febrile period (from before day 10 after onset) to late convalescence (after day 30 from onset) did not differ significantly from that of febrile or afebrile child controls (Fig. 1). There was a tendency for polymerase activity in supernatant fluids from lymphocyte cultures from KS patients and febrile controls to be slightly higher after seven to 20 days in culture; but the mean was eightfold less than the mean for cells (MT-4, Hut-102) transformed by HTLV-I, a tightly cell-associated virus (Fig. 2). There was also little or no reverse transcriptase activity in cultures from afebrile convalescent patients (>10 days after onset) during any period of culture. In afebrile controls, reverse transcriptase activity was minimally elevated during the first four days of culture (Fig. 3).

In 13 cultures from 10 patients, DNA polymerase activity measured by dTMP incorporation was assayed using additional template:primers, poly(dA)·oligo(dT) and poly(rC)·oligo(dG), to determine whether the observed DNA polymerase activity was attributable to cellular DNA polymerases or to retroviral reverse transcriptase. In each case, the poly(dA)·oligo(dT) template:primer yielded values equal to those with poly(rA)·oligo(dT), suggesting that the low DNA polymerase activity was due to host cell enzymes. Furthermore, there was no activity detected with poly(rC)·oligo(dG), a template:primer preferred by RNA-dependent DNA polymerase[5]. Therefore, we were unable to demonstrate that the observed low-level DNA polymerase activity in supernatant fluids from lymphocyte cultures of KS patients was of retroviral origin.

Lymphocytes from five acute KS patients were also co-cultured with human peripheral blood lymphocytes, brain endothelial cells, and cell lines of T-lymphocytic (Hut-78, Molt-4, C8166, HPB-ALL), B-lymphocytic (Raji), and monocytic (U-937, BT4A3.5) lineage. In no case was RNA-dependent DNA

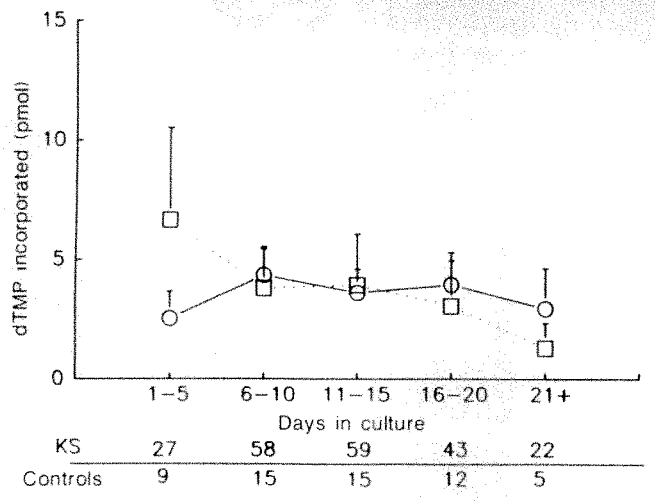


Fig. 3 Particulate polymerase activity in supernatant fluids of lymphocyte cultures. (○), KS patients in convalescent (afebrile) phase; (□), afebrile controls. The number of cultures tested at each time interval is shown on the horizontal line. Vertical brackets indicate 95% confidence intervals.

polymerase activity detected in the co-cultures. In one case however, syncytium formation was observed in a co-culture from HPB-ALL. Electron microscopic examination of this culture showed viral particles, subsequently identified as measles virus by specific immunofluorescence and radioimmunoprecipitation assays. The patient had been vaccinated with measles–mumps–rubella trivalent vaccine 8 days before onset of disease. It was concluded that the agent was measles vaccine virus.

Using standard methods for reverse transcriptase detectio we have been unable to demonstrate a retroviral aetiology f KS. The level and pattern of DNA polymerase activity detecte in KS patients in our study were similar to those previous reported[2]. In contrast to their study, cultures from our contro had values and patterns of activity similar to cultures from o KS patients. In addition, the low-level DNA polymerase activit detected in our cultures showed a template:primer preferenc consistent with cellular DNA polymerase and inconsistent wit retroviral RNA-dependent DNA polymerase. Moreover, w have been unable to detect reverse transcriptase activity i repeated co-cultures of KS patients' lymphocytes with numero human cell lines. Our experience with recovery of measles vir and its demonstration within cultured lymphocytes by electro microscopy emphasizes the danger in assuming that agen recovered from patients with KS may have a causative role.

Therefore, we believe that the aetiology of KS remair unknown. If a retrovirus is involved in the causation or th pathogenesis of this disease, techniques more sensitive than th assays presently available will be required to establish i presence.

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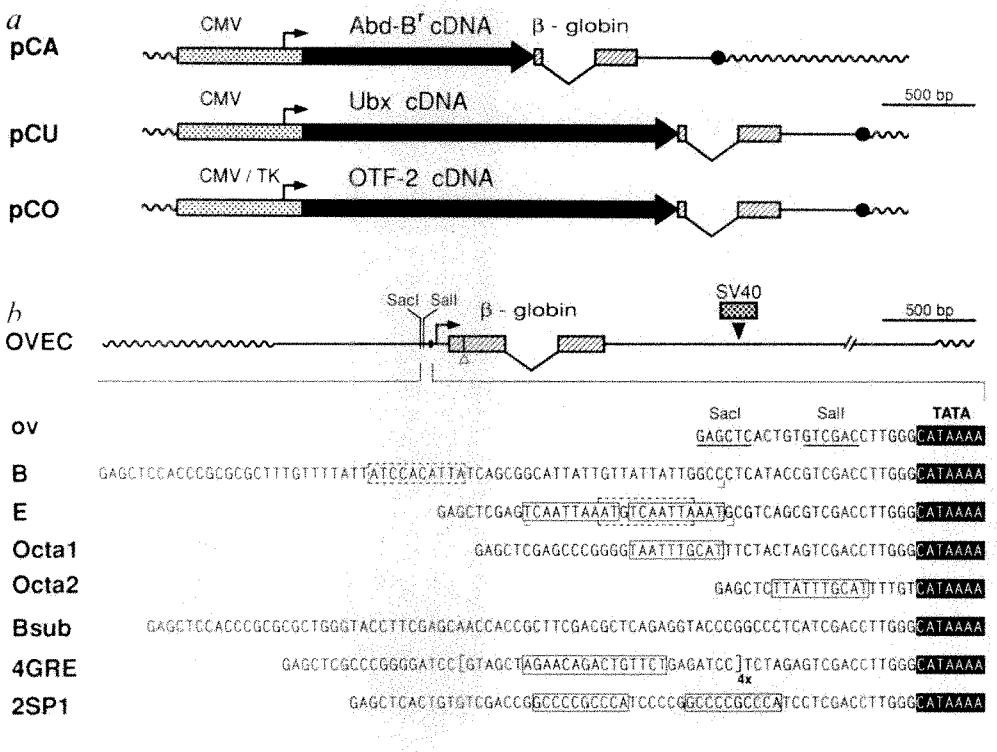
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Erratum: *Drosophila* homoeotic genes encode transcriptional activators similar to mammalian OTF-2

Markus Thali, Michael M. Müller, Mauro DeLorenzi, Patrick Matthias & Mariann Bienz

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IN this biological letter, a unrevised version of Fig. 1 wa printed. The correct version i shown here.

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POSTDOCTORAL POSITION

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N6A 5B8

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(NW3157)A

LABIMAP 2001 is one of the most exciting and potentially valuable projects current within European Molecular Biology. So it comes as no surprise that Amersham – along with the ICRF – is spearheading the UK operation, working in close collaboration with two leading French companies.

DEVELOPMENT PROJECT LEADERS

Cardiff

up to £25,000

The project's importance is such that it has gained "Eureka" status – and thus the backing of both the British and French governments. Its aim is to develop automated systems for all the major procedures used in molecular biology laboratories, making a significant contribution to, among other things, the mapping of the human genome.

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A broad mastery of molecular biology is clearly essential, as is a proven track record in research or development and an understanding of the contribution instrumentation and software techniques can make. More importantly, however, we are looking for people who, as well as a strong technical background, have the strong leadership qualities to motivate a team towards significant results, while continuing to make their own full practical contribution.

To attract individuals of the right calibre we can offer salaries of up to £25,000, plus full relocation expenses if required, and a wide range of benefits.

Please write, quoting ref. CR2 and enclosing full career details, to Bill Buckley, Personnel Manager, Research Products, Amersham International plc, Cardiff Laboratories, Forest Farm, Whitchurch, Cardiff CF4 7YT. Telephone Cardiff (0222) 615858.

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(8165)A

MOLECULAR VIROLOGIST

A staff position is available for a person with experience in the molecular biology of primate viruses. The successful candidate would be expected to become involved with research programs involving simian immunodeficiency disease as well as to establish his/her own area of research. Ongoing research includes studies investigating the interrelationships of opportunistic viruses with Simian Immunodeficiency Virus. The applicant would be expected to obtain research support as well as to work collaboratively with other investigators. Please send résumé, a summary of research interests, and the names of three references by Feb. 15 to: **Dr. Michael Murphey-Corb, Delta Regional Primate Research Center, Three Rivers Road, Covington, LA 70433.** (NW3159)A

CORIELL INSTITUTE FOR MEDICAL RESEARCH

Two Ph.D. staff positions available for a human geneticist, any discipline, and a molecular cytogeneticist. Appointee will develop independent research programs and provide technical and administrative support for the Human Genetic Mutant Cell Repository. Development funds and joint medical school appointments are available. Newly constructed laboratory space and technical assistance will be provided. Salary commensurate with qualifications.

Please send curriculum vitae and names of three references to: **Dr. Gerard J. McGarrity, President, Coriell Institute for Medical Research, Copewood St., Camden, NJ 08103.**

AA/EOE.

(NW3145)A

EUROPEAN SOUTHERN OBSERVATORY

The **European Southern Observatory (ESO)** is an intergovernmental organisation, responsible for astronomical research in the southern hemisphere. The eight member states of the ESO are Belgium, Denmark, France, Italy, The Netherlands, The Federal Republic of Germany, Sweden and Switzerland. The ESO occupies a leading position in the world scientific community as the first European astronomical centre. Its research is of use for a wide range of sciences as well as industry.

ESO is looking for a

EXPERIMENTAL PHYSICIST/ ASTROPHYSICIST

(ref. EPD302)

to start working as soon as possible in the High Resolution Imaging and Interferometry Project Team of the Project Division at the ESO Headquarters in Garching bei München, the Federal Republic of Germany. The major responsibility of this Division is the implementation of the Very Large Telescope (VLT), consisting of an array of four 8 metre telescopes.

Assignment:
To assume a major role in the realization of the interferometric imaging mode and adaptive optics of the VLT. To a large extent this will involve interaction and coordination with related activities in ESO member countries. Other activities may include the upgrading of the high resolution capabilities of the present ESO La Silla telescopes as well as the carrying out of a modest personal research program.

Experience and knowledge:
The candidate should have a university degree in physics, astrophysics or equivalent. Experience in the design, specification, and commissioning of sophisticated precision instrumentation. Familiarity with physical optics and precision optomechanical control systems is required. Knowledge of astronomical interferometric imaging techniques will be an asset as will be a demonstrated skill in the coordination and management of projects.

Remuneration:
The remuneration for this post is commensurate with the background, experience and family status of the candidate. The basic monthly salary (tax-free) will be between DM 4.600,– and DM 8.300,–. To this may be added a non-residence allowance of either 9% (single) or 12% (head of family) of the basic salary as well as some other allowances.

Although preference will be given to nationals of the Member States of ESO, no nationality is a priori excluded.

Applications, indicating the reference number, should be submitted **before February 28, 1989 to:**
Personnel Service,
European Southern Observatory,
Karl-Schwarzschild-straße 2,
D-8046 Garching near Munich, Federal Republic of Germany.
Tel.: (89) 320.06.216-8. (W5762)A

INSTITUTE OF PLANT SCIENCE RESEARCH CAMBRIDGE LABORATORY

Molecular Biologist

The Laboratory is seeking to appoint a Post-doctoral Fellow to join a group interested in gene expression during pollen development in maize. The person appointed will be expected to characterise one or more promoter elements which exhibit post-meiotic specificity in maize and to develop reporter gene constructs which can be transferred into tobacco. This position would be suited to someone with some experience in cell or molecular biology and with an interest in studying gene expression using the technique of 'in situ' localisation.

The position is funded by pioneer Hi-Bred International Inc. for a period of three years.

Initially the appointee will be located at the Cambridge Laboratory, but in March 1990, he or she will move to new laboratories at Norwich.

Salary will be on the Higher Scientific Officer scale £10,026-£13,460.

Applications with curriculum vitae and the names of three referees should be sent to **Mrs L Cliff, Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB quoting reference MG/346 by 10th February 1989.**

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Women applicants are strongly encouraged.

Applications with supporting documents and a short summary of research perspectives should be sent before **March 30, 1989** to: **the Dean, "Mathematisch-Naturwissenschaftliche Fakultät Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40 2300 Kiel 1", Federal Republic of Germany.** (W5756)A

UNIVERSITY OF BASEL
Department of Physiology Neurobiology

Applications are invited for a two year Postdoctoral position, starting August 1, 1989, to work on regulation of nicotinic acetylcholine receptor expression in skeletal muscle. Prolongation up to 4 years will be possible. Background in biochemistry of cell biology desirable. The position requires eight hours per week of teaching, in German, laboratory courses in Physiology to medical students. Salary range is sFr.50,000 to 70,000, commensurate with experience. Applications and names of two referees should be sent as soon as possible to:

Dr. H.R. Brenner,
Department of Physiology,
University of Basel,
Vesalgasse 1,
CH–4051 Basel,
Switzerland
Phone: (061) 25.40.42

(W5757)A

UNIVERSITY OF LIVERPOOL

CANCER RESEARCH CAMPAIGN
Department of Radiation Oncology

Applications are invited for the non-medical post of J.K. Douglas Lecturer (Special Appointment) in Molecular Biology.

The successful applicant will be expected to interact with a group of 4 scientists investigating the potential of anti-sense oligonucleotides to inhibit oncogene expression, which has just commenced a 5 year Cancer Research Campaign programme grant. In addition the applicant would be able to develop his/her own interests in the molecular biology of cancer cells.

Initial salary on the Lecturer A or B scale (£9,260–£19,310 per annum). Grade III technical support and some recurrent expenditure will be available during the first two years of the appointment to enable the appointee to become rapidly productive.

The Department of Radiation Oncology is situated at Clatterbridge Hospital between Chester and Liverpool in a busy oncology department. Being close to both North Wales and the Lake District it offers excellent recreational opportunities.

Informal enquiries may be made to Professor Hilmar Warenius or Dr. David Tidd (051-334 7605).

Applications, together with the names of three referees, should be received not later than 20th February, 1989, by the **Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX** from whom further particulars may be obtained. Quote Ref. RV/231/N.

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THE UNIVERSITY OF WESTERN AUSTRALIA
(Perth)
LECTURER IN GEOPHYSICS
Department of Geology
(Ref. A03/89)

ed term appointment for three irs (may be extended). The ointee will be the only geo-sicist on a teaching staff of ten, l will be responsible for under-duate teaching in general and loration geophysics. The app-tee will be expected, also, to lertake personal research, and supervise honours and post-duate research projects. It is ed that the appointee will build a geophysical research group. plications are invited from sons in all areas of geophysics, it is hoped that the research erests of the appointee will vide opportunity for co-opera-n and interaction with other mbers of the Department, e.g. such fields as continental struc-e and development, or geo-sics applied to tectonics or in physical explorations. The partment has some geophysical ipment (gravity meter, seismic raction unit, X-ray scintillo-ter, long-period recording smography).

Commencing Salary: A$30,737–33,414 p.a.

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Written applications quoting erence number, telephone mber, date of birth, qualifica-ns and experience, and the mes and addresses of three erees should reach the Direc-, Personnel Services, The iversity of Western Australia, dlands 6009, Australia, by *17 bruary 1989*.

EQUAL EMPLOYMENT OPPORTUNITY IS UNIVERSITY POLICY
(W5760)A

Pharmacologist
Analgesics Research
Zambeletti Research Laboratories

Zambeletti Research Laboratories are part of Beecham Group plc, a leading research-based International Pharmaceutical Company.

We wish to recruit a research biologist for a senior position within our Analgesic project. A minimum of 5 years' research experience in CNS pharmacology, and/or electrophysiology is desirable.

The position will appeal to a scientist in late twenties or early thirties, with a record of innovative research. We need someone who is ready for the opportunity to develop new approaches to the discovery of analgesic compounds and bring them to fruition in a stimulating professional environment. Experience in the pharmaceutical industry, whilst desirable, is not essential. The work will involve liaising with scientists in other Beecham research units in the UK and Europe.

The Zambeletti Research Laboratories are situated at Baranzate, in an attractive part of Italy on the northern outskirts of Milan, near the Alps and the Lakes. A competitive salary will be offered and relocation assistance will be provided. Fluency in Italian, whilst desirable, is not essential.

Applicants should send a curriculum vitae to Dr. V. Vecchietti, Zambeletti Research Laboratories, 20021 Baranzate, Milan, Italy.

Beecham Pharmaceuticals

(8141)A

POSTDOCTORAL POSITION

Available from April 1989 to work on regulation of expression and function of the c-*mos* proto-oncogene. Applicants should have a molecular biology training and an interest in mouse embyogenesis. Send curriculum vitae and names of three referees to:— **Dr. F. Propst, Ludwig Institute for Cancer Research, St. Mary's Hospital Medical School, Norfork Place, Paddington, London W2 1PG (Telephone 01 724 5522).** (8130)A

AFRC INSTITUTE OF FOOD RESEARCH
PROTEIN CHEMIST

A protein chemist/biochemist is required to design and synthesize peptides with novel emulsification properties. The peptides will have desired functional properties in fat-water systems of both theoretical and commercial importance. Biochemical and biophysical techniques will be used to determine how they, and muscle proteins, bind and function at interfaces. A practical knowledge of protein synthesis and sequencing is essential.

Qualifications: 1st or upper 2nd class honours degree in biochemistry, biophysics or related subject with a sound background in protein chemistry. A PhD in protein chemistry, biochemistry or biophysics an advantage.

Salary: £12,445–17,032 pa.

Application forms from: The Personnel Office (Ref. 88/13) Institute of Food Research, Bristol Laboratory, Langford, Bristol BS18 7DY. Closing date 6th February 1989.

The AFRC is an equal opportunities employer. (8132)A

UNIVERSITY OF WARWICK
Research Technician
Molecular Biology

The Department of Biological Sciences has two vacancies for MRC funded Research Technicians to work within the Virology research Group. The projects involve the study of either a human adenovirus protein involved in RNA metabolism or the molecular mechanisms of pathogenesis in mammalian reoviruses.

The contract posts are available for up to three years.

Experience of recombinant DNA techniques and tissue culture would be an advantage, but interest in the project and an enthusiasm to learn new techniques are equally important. Full training will be given where necessary. Salary on the Technician Grade 3 scale: £6,636–£7,828.

Application forms from the **Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523685) quoting Ref No 23/4T/88** (please mark clearly on envelope). Closing date for applications 25 January 1989.

An Equal Opportunities Employer. (8123)A

KING'S COLLEGE SCHOOL OF MEDICINE & DENTISTRY of King's College London
Post-Doctoral Research Assistant in Pharmacology

This position, suitable for a recent doctoral graduate, is to investigate the pharmacology of lymphocytes in allergic asthma. The position is available for two years and the successful candidate will be required to work with Dr. C. Page (King's College London, Chelsea Campus) in collaboration with Dr. J. Costello (Thoracic Medicine, King's College School of Medicine and Dentistry).

Salary will be on the scale £9865 – £15720 plus £1650 London Weighting Allowance per annum.

Further details are available from Dr. C.P. Page, Department of Pharmacology, King's College London, Manresa Road, London, SW3 6IX. (Telephone: 01-351 2488 ext. 2583).

Applications, including a full curriculum vitae and the names of two referees should be sent to the **Secretary of the School, Bessemer Road, London SE5 9PJ by 8th February 1989.** (8108)A



La Trobe University
Australia
School of Physical Sciences
SENIOR RESEARCH ASSISTANT IN PHYSICS

(Fixed term)

A Research Associate (Experimentalist or Theorist) is required to work on the irregular structure of the ionosphere at mid-latitudes. The appointee will be a member of the group consisting of E.C. Butcher, K.D. Cole, P.L. Dyson, E.A. Essex and P.R. Hammer. The group has a Digisonde 256, Fabry-Perot interferometer, Faraday polarimeters, Differential Doppler receivers, HF Doppler system, geomagnetic pulsation recorder and related equipment. The position will be funded by the Australian Research Council for one year initally.

Closing Date: 28 February, 1989. Ref. No.: ACA 5/753.

Salary: $A26,617 - $A28,118.

Applications (marked confidential) including reference number, names and addresses of three referees and curriculum vitae should be forwarded to the Staff Officer, La Trobe University, Bundoora, Victoria, Australia, 3083. (W5765)A

Equal Opportunity is University Policy. arm42189

SCHOOL OF PHARMACY
(UNIVERSITY OF LONDON)
TEMPORARY LECTURER IN PHARMACOLOGY

Applications are invited for the post of Temporary Lecturer in Pharmacology, tenable for two years with effect from 1 October 1989. The appointment will be at the Lecturer A grade (salary in the range £9,260-£14,500 + £1,650 London Allowance). Prospective candidates are welcome to discuss the position informally with Professor N G Bowery (01-837-7651 Extn 31).

Applications in the form of a Curriculum Vitae and naming two referees should be submitted to **Mr N J Rampley, Assistant Secretary, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX** by no later than the end of February 1989. (8129)A

PROFESSORSHIP IN SEDIMENTARY GEOLOGY
University of Mainz, West Germany

The Department of Geosciences, University of Mainz, invites applications for a tenured professorship in sedimentary geology (C-3 level, equivalent to senior lecturer in UK and associate professor in US) beginning April 1st, 1989.

Duties include teaching (in German) in introductory geology, in the wider fields of sedimentology, and supervision of student at MS and PhD levels. The successful candidate is expected to develop an active program of grant-funded research in the field of clastic sediments with focus on facies and basin analysis, and with special emphasis on sequence stratigraphy. Cooperation in a planned interdisciplinary research project focussed on various topics of crust-mantle evolution is expected. Applications of candidates with additional interests, e.g. in environmental geology, are particularly welcome.

A PhD is required, and preference will be given to candidates with teaching experience and an outstanding record of research in sedimentology.

Please submit full curriculum vitae, 3 references, publications and a statement of teaching and research interests to the **Dekan, Fachbereich Geowissenschaften, Universität Mainz, Postfach 3980, D-6500 Mainz, F.R.G.** Applications should be submitted by Feb. 28th, 1989.

(W5758)A

PROTEIN CHEMIST
RUAKURA AGRICULTURAL CENTRE
MAF, HAMILTON, NEW ZEALAND
VACANCY NO. MTN 88/24

The Ruakura Animal Reseach Group of the Ministry of Agriculture & Fisheries has an opening for a protein chemist. The appointee will be required to develop and extend existing programmes in animal physiology research including growth and lactation physiology, reproduction and disease control.

The successful candidate will have proven expertise in the contemporary technology of protein chemistry and have demonstrated the ability to communicate and collaborate with associates in a multi-disciplinary team. Specific skills in hybridoma ELISA and immunocytochemistry technologies and experience in growth factor biochemistry or endocrinology would be an advantage.

The minimum qualifications are an honours degree and PhD and preference will be given to applicants with post-doctoral experience.

Hamilton is a medium sized community (popn 100,000) set in farmland with excellent access to all forms of outdoor recreational activities.

Further information can be obtained from Mr Ken Jury, Manager Animal Sciences, Ruakura Agricultural Centre, Private Bag, Hamilton, New Zealand; Telephone (+64 71) 62839; Fax (+64 71) 385 658.

Applications quoting vacancy number MTN 88/24 and including a curriculum vitae and the names of two referees should be sent to:

Management Support Officer
Animal Research Group
MAFTech
Ruakura Agricultural Centre
Private Bag
Hamilton
NEW ZEALAND

Applications for vacancy close on 28 February 1989. (W5763)A

POSTDOCTORAL POSITION

in biology at M.I.T. available immediately for studies on translational control in eukaryotes with emphasis on regulation of protein chain initiation by phosphorylation-dephosphorylation mechanisms. Specific aspects focus on the roles of dsRNA-activated and heme-regulated eIF-2a kinases, and on the function of 'reversing factor' in the recycling of eIF-2. Contact: **Dr. Daniel Levin, Building E25-519, Massachusetts Institute of Technology, Cambridge, MA 02139.** Telephone: 617-253-4307. (NW3154)A

NORTH STAFFORDSHIR HEALTH AUTHORITY
SENIOR RESEARCH SCIENTIST IN RHEUMATOLOGY

Department of Post Graduat Medicine, University of Kee

£14,389–£19,895 pa

This is a new post develo between the Department of F Graduate Medicine, Universit Keele and the Staffordsh Rheumatology Centre and fun for a period of 3 years. The incu bent will head and develop labo tory research into the immu inflammatory mechanisms rheumatoid diseases and will accorded appropriate acade status according to qualificati and experience. Applications invited from scientists with prov research experience from wit any of the biological sciences.

For Job Description, furth information and arrangements visit, please apply to: Dr P Dawes, Consultant Rheumat ogist, Staffordshire Rheumatol Centre, Haywood Hospital, Hi Lane, Burslem, Stoke on Tre ST6 7AG. Tel: (0782) 835721 E 302.

Applicants should submit a f curriculum vitae, plus the nam and addresses of three referees Dr P T Dawes by 28 Februa 1989. (8117)A

UNIVERSITY OF NOTTINGHAM
Medical Research Technicians (2 posts)

Applications are invited from men and women for the above posts in the Department of Therapeutics. One project, for which experience with radioimmunoassays and/or prostaglandin compounds will be important, will investigate the effects of smoking and analgesic drugs on the gastrointestinal tract. The second project, for which experience with HPLC will be important, will investigate the effect of fatty acids on the metobolic activity of the colon.

Minimum qualifications required HNC, Higher TEC or equivalent.

Current salary scale, under review, £6,686 to £9,435 per annum for a 5 day, 37 hour working week.

These are research funded posts which will be initially offered for one year.

Apply in writing, enclosing curriculum vitae and the names of two referees to **Mr. C. R. Richmond, Department of Therapeutics, University Hospital, Queen's Medical Centre, Nottingham, NG7 2UH.** (8135)A

Senior Scientist

GLAXO GROUP RESEARCH LTD.
virology department

&

MRC COLLABORATIVE CENTRE

A Joint Venture - HIV Research

Glaxo is Britain's largest and one of the world's most successful pharmaceutical companies. Our commitment to research and development of new therapeutic agents has made us the world's second largest ethical pharmaceutical company.

The Collaborative Centre operates as a technology transfer establishment designed to provide laboratory facilities for the testing of early research ideas. It also houses MRC funded projects with highly directed commercially important objectives, such as those within the MRC AIDS Directed programme.

A unique permanent vacancy has been created for a Senior Research Scientist to work in the Virology Department of Glaxo Group Research, on secondment to the MRC Collaborative Centre, to develop techniques for the assessment of anti HIV agents, and study their mode of action. You will also supervise the work of a scientist engaged on the same project.

You will have a PhD or equivalent experience, and a proven research record in virology, or a related discipline with expertise in mammalian cell culture, and preferably experience of working with HIV.

In return, you will receive an excellent salary and guaranteed bonuses, (salary range from 15k to 21k inclusive) plus a wide range of benefits including a non-contributory pension scheme, subsidised health care and restaurant, as well as an active sports and social club.

Please send a CV or telephone for an application form to Fiona David, Personnel Officer, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE (01-422 3434 ext 2934) quoting reference number G88324.

(8105)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

SENIOR RESEARCH OFFICER (postdoctoral): cell biology/immunology

Applications are invited from postdoctoral scientists for an MRC funded position in an interdisciplinary research group studying the regulation of cellular function in asthma. The successful candidate will work within the asthma group, and be primarily responsible for investigating the effect of anti inflammatory and anti allergic drugs on both the expression of the low affinity IgE receptor (the CD23 antigen) and the transduction of the evoked signal. Applicants should have a Ph.D. in biochemistry, immunology, pharmacology or a related discipline with a research background in cell biology.

The post is funded on an MRC programme grant until May 1991.

Salary on Range 1A.

Informal enquiries to Dr R J Fuller or J MacDermot (01-740 3220).

Application forms and further particulars from **the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN (tel: 01-740 3204)** quoting reference AEB2/SRO.

Closing Date: 3rd February 1989. (8109)A

RESEARCH ASSISTANT

required to participate in a research programme funded by the Medical Research Council until September 1993. The project is concerned with the analysis of genes affecting pattern formation and cell orientation in *Drosophila*. The duties of the post will include the extraction and purification of DNA from plasmid cultures, sequencing, cloning and nick transplantations using various biochemical techniques.

Salary £8675-£11680 depending on age.

Applications, to include c.v and the names of two referees, to: **Dr D. Gubb, University of Cambridge, Department of Genetics, Downing Street, Cambridge CB2 3EH. Telephone 333999.** (8111)A

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
Department of Neuroscience

POSTDOCTORAL MOLECULAR BIOLOGIST

Applications are invited for the above post to join a group studying genetic factors involved in the causation of mental illness. The post involves the study of brain gene expression in man and the development of model systems to investigate gene systems involved in psychotic states. Experience in mRNA analysis/cDNA cloning techniques is preferable but not essential.

The appointment will be for 16 months in the first instance, with salary on an appropriate point on the 1A scale in the range £9865-£14500 pa plus £1650 London Allowance.

Further information may be obtained from Dr S Whatley on 01-703-5411 ext 3263.

Applications in the form of a curriculum vitae plus the names of two referees should be sent to the **Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF quoting reference SW/N.** Closing date: 10th February 1989. (8112)A

CORIELL INSTITUTE FOR MEDICAL RESEARCH

Two Ph.D. staff positions available for a human geneticist, any discipline, and a molecular cytogeneticist. Appointee will develop independent research programs and provide technical and administrative support for the Human Genetic Mutant Cell Repository. Development funds and joint medical school appointments are available. Newly constructed laboratory space and technical assistance will be provided. Salary commensurate with qualifications.

Please send curriculum vitae and names of three references to: **Dr. Gerard J. McGarrity, President, Coriell Institute for Medical Research, Copewood St., Camden, NJ 08103.**

AA/EOE. (NW3145)A

Department of Anatomy & Developmental Biology
UNIVERSITY COLLEGE LONDON

Post-doctoral Research Assistant required for project concerned with the expression of genes involved in controlling the primary phenotypic differentiation of neurones during early neural development. Initially the project will focus on sodium pump genes, for correlation with physiological experiments showing that functional activation of the Na pump is an essential part of neural differentiation. The molecular work will complement experiments using electrophysiological, tissue culture and immunohistochemical techniques. Previous experience of molecular techniques is essential.

The appointment is supported by a grant from the Wellcome Trust and will be on the 1A range, with salary according to age and experience. Starting date by arrangement asap after Jan. 1st 1989. Applications (including c.v. and two referees) should be sent to **Professor A. Warner, Dept. of Anatomy & Developmental Biology, University College London, Gower St., London WC1E 6BT,** from whom further particulars may be obtained (Tel: 01-387-7050, Ext 3359). Closing date 3 weeks after appearance of this advertisement. *Equal Opportunities Employer.* (8115)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL POSITION

A cell biologist or biochemist is required to join a team working on the biochemistry and genetics of mammalian cell DNA repair, under the direction of Dr Andrew Collins. The project will involve the production and characterisation of mutant cell lines defective in DNA replication or repair. Candidates should have a PhD and experience in mammalian cell culture.

The position is funded by the Cancer Research Campaign, for two years, with salary in the range of £9,865 – £11,680 on the IA Scale for Research and Analogous Staff. **Application forms available from the Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX (tel 0224 273500), to whom applications (2 copies) should be returned by 10 February 1989 quoting reference number DA/046.** (8116)A

INSTITUTE OF CANCER RESEARCH
ELECTRON MICROSCOPY — TECHNICIAN

The Institute of Cancer Research has a vacancy for a technician to work in its electron microscopy laboratories based at Sutton in Surrey. The laboratory provides an ultrastructural service to the Institute covering both transmission and scanning electron microscopy, with a considerable commitment to ultrastructural immunocytochemistry. In addition, the laboratory has a strong research programme on the structure of normal and neoplastic human breast epithelium.

Previous experience in electron microscopy would be highly desirable for this post, and there will be the opportunity to gain experience in both cryo-sectioning and freeze substitution.

Salary in the range of £7881 to £10711.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Further information on the past may be obtained from Dr. P. Monaghan on 01 643 8901 ext. 4631.

Applications should be sent in duplicate with the names and addresses of two referees to the **Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL,** quoting reference number 1 89.T.N.62. (8131)A

THE INSTITUTE OF CANCER RESEARCH
Fulham Road, London
RESEARCH OFFICER

A Research Officer is required to join a team working on the mechanism of action of interleukin 2. The applicant must have experience in laboratory work, and preference will be given to candidates with knowledge of protein chemistry and/or mammalian cell culture. A theoretical and practical grounding in immunology is desirable.

Salary scale £7,953 to £11,162 inc. according to experience.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

To apply, please forward a CV with the names and addresses of two referees, to the **Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London, SW7 3AL,** quoting ref. no. 12.88.T.NS.55. (8134)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
DEPARTMENT OF HAEMATOLOGY
TECHNICIAN

Required for research unit within the Department of Haematology. The successful applicant will be expected to spend a proportion of his/her time working on an MRC funded project on the "Molecular Genetics of G6PD" whose principal investigators are Professor Lucio Luzzatto and Dr Philip Mason. Other duties will include looking after equipment and supplies in a busy research laboratory with numerous Fellows and trainees.

Whilst a full time person is preferred, consideration will be given to maximum part-time applicants if highly experienced. Informal visits to the laboratory from suitably qualified people will be welcome.

Application forms and further particulars may be obtained from the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN (tel: 01-740 3204)** quoting ref: AFA2. Closing Date: 9th February 1988.

Salary on range £7,415–£10,148 + £1,267 London Allowance per annum. (8146)A

UNIVERSITY OF OXFORD
University Laboratory of Physiology
POSTDOCTORAL NEUROPHYSIOLOGIST

A postdoctoral position is available, funded by the Wellcome Trust for up to 4 years, to study cortical processing of somatosensory information from the primate hand. Experience with extracellular recording techniques, animal taining and computer analysis of neurophysiological data would be an advantage. Further information may be obtained from Dr. John Phillips, University Laboratory of Physiology, Oxford 272511.

Salary (Research Assistant Grade 1A), £9,865-£15,720 p.a.

Written applications, including a CV and the names and addresses of two referees, should be sent to the **Administrator, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT.**

The University is an Equal Opportunity Employer. (8145)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
World Health Organisation collaborating centre for Reference and Research on Viral Hepatitis
RESEARCH FELLOW IN BIOCHEMISTRY OR VIROLOGY
Department of Clinical Sciences

Applications are invited from post-doctoral scientists with experience in protein bio-chemistry to work with a small group headed by Professor A J Zuckerman on the development of novel vaccines against AIDS. Appointment for three years on salary scale 1A for Research and Analagous staff (£11,315 to £17,170 inclusive of London Weighting).

Please send curriculum vitae and names of two referees to **Peter Guildford, Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT or telephone 01-636 8636 ext. 201** for an application form. Closing date 6 February 1989. (8147)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
UNIVERSITY OF LONDON
DEAN

The School is seeking a successor to Dr Bruce MacGillivray, who retires from the post of Dean in the Autumn of 1989. The new Dean must be medically qualified. He or she will direct the School's internal affairs, maintain and develop its relationship with the University, and further the existing interactions with the Regional and District Health Authorities.

Confidential enquiries, suggestions and recommendations are invited, and should be addressed to **Dr C.W.H. Havard (Chairman of the Search Committee), Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF,** by 10 February 1989. (8153)A

ANIMAL ECOLOGIST ENDOWED CHAIR IN VETERINARY MEDICINE

ANIMAL ECOLOGIST is sought with experience preferably in domestic animal species and their interaction with humans for a tenure-track faculty position and endowed chair in the School of Veterinary Medicine, University of Pennsylvania. Additional background may include ethical issues, the use of animals, and insight into the ethical belief system of the veterinary profession. Candidates should have credentials of innovation and academic leadership. Address inquiries to:

Dr. K.C. Bovee, Chairman
Moore Chair Search Committee
School of Veterinary Medicine
University of Pennsylvania
3850 Spruce Street
Philadelphia, PA 19104-6010

The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer. (NW3148)A

ENDOWED PROFESSORSHIP
AT OREGON STATE UNIVERSITY
VALLEY CHAIR IN MARINE BIOLOGY

Oregon State University

The Department of Zoology at Oregon State University invites applications and nominations for the *Wayne and Gladys Valley Professorship in Marine Biology*. We expect the successful candidate to contribute substantially to research and graduates training in some aspects of marine biology. Applicants should have an established international reputation in marine biology, including a strong research program with evidence of continued growth and development. Teaching opportunities may include a graduate course in the candidate's specialty and participation in a Marine Biology course, team-taught each spring at the Hatfield Marine Science Center (HMSC) in Newport, Oregon. Research space and facilities are located on the main campus in the Department of Zoology, but we encourage research activity at the HMSC, where additional research facilities and resources are available.

Oregon State University has considerable strength in the marine sciences, including scientists in the Colleges of Science (Zoology, Botany and Plant Pathology, Biochemistry/Biophysics, Microbiology, General Science), Agriculture (Fisheries and Wildlife), and Oceanography. The Department of Zoology includes an active group of Marine Biologists with expertise in behavioural, population and community ecology, paleobiology, invertebrate zoology, ichythyology, evolutionary biology, and physiology.

Review of applications will begin February 1, 1989. Deadline for applications material is September 15, 1989. Nominations should address the relevant qualities of the nominee. Applications should include a curriculum vitae, copies of representative publications, a statement of research goals, and the names of four references sent to: **Dr. Bruce Menge, Chair, Marine Biology Search Committee, Department of Zoology, Oregon State University, Corvallis, Oregon 97731-2914.** *Oregon State University is an Equal Opportunities/Affirmative Action Employer and complies with section 504 of the Rehabilitation Act of 1973. OSU has a policy of being responsive to the needs of duel career couples.* (NW3153)A

MOLECULAR VIROLOGIST

The School of Medicine at Case Western Reserve University invites applications from new or established investigators for a program in molecular virology. We are seeking faculty members with primary interests in virology, especially in the area of human retroviruses and AIDS research. The CWRU School of Medicine provides a strong and interactive environment for collaborative research among basic and clinical investigators. Ample resources and newly renovated facilities are available for this program. Case Western Reserve University is an Affirmative Action and Equal Opportunity Employer. Interested applicants should contact:

Hsing-Jien Kung, Ph.D. and
Jerrold Ellner, M.D.,
Co-Chairmen,
Virology Search Committee,
Office of Academic Programs,
Case Western Reserve
University School of Medicine,
2119 Abington Road,
Cleveland, Ohio 44106.

(NW3041)A

HEAD, ENDOTHELIAL CELL RESEARCH

Berlex Laboratories, Inc. is a leader in basic research of endothelial cell function. We currently seek an internationally recognized scientist in endothelial cell biology to join our highly visible team to ensure progress of on-going programs, to initiate new research directions and to be part of future breakthroughs which can be applied to better understanding and novel treatment of atherosclerosis. The successful candidate will have the title of Investigator, the second highest rank in our scientific ladder.

To qualify, your background should include a Ph.D. in cell biology, biochemistry, physiology or molecular biology, at least five (5) years of post-doctoral training and an excellent publication record. Experience with endothelial cell biology is highly desirable.

Berlex Laboratories is part of a global family of companies engaged in the research, development, manufacturing and marketing of chemical-pharmaceutical products in 120 plants and subsidiaries in 140 countries, employing over 25,000 people. Combined sales are in excess of $2.7 billion.

At Berlex Laboratories, you will be supported, recognized and encouraged to pursue your scientific career and ideas. In addition to the excellent resources and stimulating research environment, the position offers an attractive salary and benefit package. For immediate, confidential consideration, send your CV and salary history to: **Lila Bernstein**, Manager, Human Resources, **BERLEX LABORATORIES, INC.**, 110 East Hanover Avenue, Cedar Knolls, NJ 07927. Equal opportunity employer M/W/H/V.

(NW3155)A

BERLEX Laboratories, Inc.



SENIOR EMBRYOLOGIST

CAMBRIDGESHIRE

Bourn Hall is a leading centre of excellence for the treatment of infertility, specialising in advanced assisted conception techniques.

A Senior Embryologist is required to work in a small team running the IVF programme. This is a key position in the embryology department with major responsibility for ensuring the quality of IVF services. The post holder will also be expected to initiate and undertake novel and innovative research in the field.

A PhD plus a minimum of three years experience in active IVF is necessary.

An excellent salary and benefits package will be offered, including BUPA, and relocation where appropriate.

If you wish to discuss this position further, please phone Dr Mike Macnamee on Cambridge 315955. To apply please send a CV to **Penelope Bucknell, Personnel Manager, Bourn Hall Clinic, Bourn, Cambridge, CB3 7TR** quoting reference SE3. (8164)A

ADVERTISEMENT

The Department of Chemistry and Biochemistry at Concordia University, Montreal

Has an opening for a tenure-track appointment at the rank of Assistant Professor in Biochemistry to commence in September, 1989. The Department has strong research interests at the interface of Chemistry and Biology in Bioinorganic, Bioorganic and Biophysical Chemistry. We are seeking an outstanding candidate with research interests in areas of Basic Biochemistry, such as; Enzymology, Metabolism or membrane Enzymes, that will compliment our existing interests. The successful applicant will have a Ph.D. and Post Doctoral experience, will be required to establish a vigorous Research Program that will attract external funding, and teach Biochemistry effectively at the Undergraduate and Graduate levels. Start-up assistance will be available from the University, while external funds are available from Quebec and federally from the Natural Sciences and Engineering Research Council of Canada. The current salary floor for Assistant Professors is $32,003.00 per year.

Concordia University is a predominantly English Institution located in downtown Montreal close to three other Research Intensive Universities. There are 29 full-time Faculty in the Department of Chemistry and Biochemistry with active Research Programs in Biochemistry, Organic, Inorganic and Physical Chemistry, Photochemistry, and Materials Science. At the present time there are 50 Graduate Students enrolled at the M.Sc. and P.hD. levels in the Department and about 15 of these are doing Research in the area of Biochemistry. The Department is housed adjacent to the Biology Department and this facilitates collaborative Research interactions between Biochemists in the two Departments.

All qualified individuals are encouraged to apply, but in accordance with Canadian Immigration requirements, this Advertisement is primarily directed to Canadian citizens and permanent residents. Applicants should supply a Curriculum Vitae, a short Resume of Research interests, a summary of start-up requirements and arrange for three letters of reference to be sent to:

Dr. P.H. Bird, Department of Chemistry and Biochemistry Concordia University, 1433 de Maisonneuve Blvd., West, Montreal, Quebec. H3G 1M8. (NW3156)A

Research Assistant/ Demonstrator

(£7,950 – £9,009 p.a.)

A Biochemist, Food Scientist or equivalent is required to work on a Food Science related project entitled "Biochemical Characterisation of Food Proteins" sponsored by the Laboratory of the Government Chemist. The research is concerned with using modern analytical techniques to identify unique biochemical characteristics of certain foods which may be used in the assessment of purity and quality.

Candidates should hold at least an Upper Second Class Honours degree in Biochemistry, Chemistry, Food Science or related subject. The person appointed will be able to register for a higher degree.

Facilites for research are excellent. Based in purpose built laboratories you will have access to the most modern equipment both at Trent and at the Laboratory of the Government Chemist.

Further details and an application form are available from the Personnel Office, Trent Polytechnic, Nottingham, Burton Street, Nottingham, NG1 4BU, Tel (0602) 418248 Ext 2148. Please quote post number S0113. Closing date 8 February 1989. (8150)A

Trent Polytechnic Nottingham

Nottinghamshire County Council

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL (University of London)

DEPARTMENT OF ANATOMY AND HISTOPATHOLOGY

Applications are invited for a research assistant's post tenable until 30th November 1990, funded by Action Research for the Crippled Child. The work involves the development of a method to treat inherited diseases of muscle using retroviral vectors to insert cloned genetic material into muscle cells of myopathic individuals. Candidates should have gained a good B.Sc. Honours degree in Cell Biology, Virology, Biochemistry, or a related subject. Some understanding of relevant DNA techniques and a working knowledge of tissue culture methods would be desirable.

Starting salary on the Research 1B scale £8,675 p.a. plus £1,650 p.a. London Allowance. Further information may be obtained from Dr. Diana Watt (01-846-7036) or Dr. T. Partridge (01-846-7149).

Applications on forms obtainable from **The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St. Dunstan's Road, London W6 8RP,** to be submitted within three weeks of the appearance of this advertisement. (Quote Ref: 89/03). (8152)A

UNIVERSITY OF WARWICK RESEARCH TECHNICIAN

Department of Biological Sciences

There is a vacancy for a Research Technician funded by the Medical Research Council as part of a comprehensive programme of research on the Molecular Genetics of Respiratory Syncytial Virus. Research is directed towards definition of gene function, identification of determinants of virulence, analysis of antigenic variability and vaccine development. Applicants should have experience or an interest in molecular biology and/or virology.

The post is available from 1 January 1989 for 5 years 9 months on Technician Grade 3 scale: £6636–£7828 pa.

Application forms from the **Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523685)** quoting Ref No 23/T/88/14 (please mark clearly on envelope). Closing date 25 January 1989.

An Equal Opportunities Employer (8142)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF SURGERY

MRC TRIAL COORDINATOR

Applications are invited from science graduates for the post of Trials Coordinator for an MRC supported trial on the surgical treatment of gastric cancer. This study is a mulitcentre one and is coordinated from an office in the Department of Surgery at Ninewells Hospital under the direction of Professor A Cuschieri.

The post which is full-time carries administrative duties (data form collection from the surgeons participating in the study, randomization to the surgical treatments and preparation of reports on the progress of the study). A limited amount of travel to other hospitals involved in the study is necessary to deal with any local problems affecting recruitment. Desirable attributes for the post include computing experience, initiative and the ability to relate and communicate with people easily.

The post will be for 3 years in the first instance. Salary will be on the Other Related Staff Grade 1 salary scale (£8,675 to £13,365), depending on experience.

Further particulars can be obtained by contacting Professor A Cuschieri at Ninewells Hospital (0382 60111 ext 2174).

Applications with full cv (2 copies) and the names of 2 referees must reach the **Personnel Office, The University, Dundee, DD1 4HN** by 3 February 1989. Please quote Ref: EST/401/89/N.

(8139)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF ANATOMY
GRADUATE RESEARCH ASSOCIATE

Applications are invited for the post of Graduate Research Associate in the Department of Anatomy, University of Edinburgh. The post is for a fixed term of three years and is concerned with the surgical repair of peripheral nerves. Previous experience of light and electron microscopy would be an advantage. The post is intended for a recent graduate seeking a career as a research associate, and it is hoped that the post will commence as soon as possible. Subject to fulfilling University requirements the successful applicant would be encouraged to read for the degree of M.Phil.

Salary on University Research Grade 1B (£8,675–£9,865).

Further particulars may be obtained from the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS,** with whom applications by letter, including c.v. and the names and addresses of two referees should be lodged by 9th February 1989. PLEASE QUOTE REFERENCE NO. 5625. (8107)A

RESEARCH ASSISTANT
MOLECULAR BIOLOGY

Pfizer has an exciting array of newly discovered agents for the treatment of animal diseases and for improving the health and productivity of farm livestock.

In order to maintain the momentum and productivity of our research programme we are now seeking to recruit a graduate with research experience in Molecular Biology to join one of our multi-disciplinary research teams investigating new approaches to the control of parasites in domestic livestock.

If you are a graduate in a biological discipline with up to five years research experience using cell or molecular biology techniques we would like to hear from you.

You will be responsible for experimental work aimed at developing new in vitro screens for antiparasitic agents and for contributing to basic discovery research.

This is a particularly exciting time to join Pfizer as we have a major multi-million pound expansion of our research facilities under construction demonstrating our long-term commitment to research here at Sandwich and enabling our scientists to look forward with confidence to the 1990's and beyond.

If you join us, you will be paid a good salary, be eligible for an annual performance bonus of up to 20% of salary, participate in our excellent employment benefit package of pension, share investment and private medical insurance schemes and be able to live in one of East Kent's charming towns or villages. Naturally, we will pay your removal expenses.

If you are interested send a brief c.v. to Mr D O'Callaghan, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ. We will take it from there.

(8148)A

NEURON IMAGING IN SLICES AND GANGLIA

at Howard Hughes Medical Institute, Department of Neurobiology, SUNY Stony Brook. Research Associate/ Research Assistant Professor positions for electrophysiologists interested in ion channels, confocal microscopy, calcium imaging, and synaptic function. Send curriculum vitae and three references to:

Dr. Paul Adams, Department of Neurobiology, Stony Brook, N.Y. 11794.

(NW3152)A

HERIOT-WATT UNIVERSITY & THE CAMBRIDGE IRC
RESEARCH IN HIGH TEMPERATURE SUPERCONDUCTORS

Applications are invited for a postdoctoral position of two years duration to work on the synthesis, structural characterisation by neutron and X-ray diffraction, and electrical measurements on new mixed valence cuprates of relevance to high temperature superconductors.

The successful applicant will join a collaborative programme between Dr Bruce and Professor Harper at Heriot-Watt and Dr Edwards at the Cambridge IRC. Experience in solid state chemistry or crystallography is not essential. Salary will be on the normal RA 1A scale £9867-£15720 with USS.

Please send CV and the names of two referees to **The Staff Office, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS** quoting ref no 2/89/N as soon as possible. Preliminary enquiries and further details may be obtained from Dr P G Bruce, tel: 031-451 3107. (8120)A

DISCOVERY BIOLOGY ANIMAL HEALTH

Section Head — to £30,000 + car

Our Client is one of the world's largest animal health businesses with a broad line of products primarily for the cattle, swine and poultry sectors. Their continued success can be attributed to the Company's commitment to original research which results in an enviable succession of novel and commercially viable agents to improve the productivity and health of farm livestock.

An unusual opportunity has now arisen for a talented research scientist to join the UK Animal Health Group to lead a well established team of scientists engaged in discovery biology, primarily concerned with parasitology, but including exploratory projects in other fields.

Ideal candidates will have a good first degree in a biological discipline followed by a PhD, preferably in Parasitology. Clearly there must be a proven record of scientific achievement and it would be a distinct advantage if some of the experience had been gained in industry orientated towards animal health.

Salary is certainly not likely to prove an obstacle and there is an excellent benefits programme which includes a car and relocation expenses.

This is an equal opportunity appointment and those interested in receiving further details should contact Roger Bryant or Martin Duchesne at:

Search And Selection Limited
Management Consultants
Suite 3, Highway House, 17 London End,
Beaconsfield, Bucks HP9 2HN Tel: 0494-671651 (8126)A

NATIONAL INSTITUTE OF
BIOLOGICAL STANDARDS AND CONTROL

HEAD OF BIOLOGICAL SERVICES

Applications are invited from senior scientists for the position of Head of the Institutes Biological Services Section.

The Institute is responsible for the standardization and control of biological substances used in human medicine and is a major WHO international laboratory for Biological Standards.

The section is responsible for the husbandry of a wide range of laboratory animals. As well as having managerial control of the section the successful candidate will be expected to participate in a mutually agreed field of biological research relevant to the Institutes responsibilities.

Applicants should be appropriately qualified scientists in Veterinary Science or a Biological Science, preferably with substantive management experience, gained in a similar environment.

Salary depending on qualifications and experience will be up to £26,125 per annum inclusive of London Weighting allowance.

Informal enquiries can be made to Ms M. Kelly. Details of the post and the Institute are available from the Personnel Office.

To apply, please submit a full c.v. together with the names and addresses of three referees to The Personnel Office, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Herts. EN6 3QG.
Telephone No: 0707 54753 Ext 457.

Please quote reference: AS/136

Closing Date: 9 Feb 1989

(8118)A

NIBSC

National Institute for Biological Standards and Control

2 POSTDOCTORAL POSITIONS IN MOLECULAR CELL BIOLOGY

Two positions are available immediately to study the molecular mechanisms of cell and tissue interactions which regulate cell differentiation in the embryonic visual system. Emphases are on the role of plasma membrane molecules and molecular genetics. Salary commensurate with experience.

Send C.V. and three letters of recommendation to:

Dr. Paul J. Linser
Whitney Laboratory
University of Florida
9505 Ocean Shore Blvd.
St. Augustine, FL 32086

An affirmative action equal opportunity employer. (NW3146)A

POST DOCTORAL POSITION

Available to work on molecular biology of plant defense reactions. The post is for one year in a first instance to start no later than May 1st.

Send C.V. and two references to:

Dr. P. RICCI
STATION DE BOTANIQUE ET PATHOLOGIE VEGETALE
Villa Thuret — 65 Bd. du Cap
Chemin Raymond
06600 ANTIBES
Tél. 93.67.88.65 (W5770)A

POSTDOCTORAL RESEARCH FELLOW

BIOMOLECULAR MODELLING LABORATORY

Applications are invited for a three year postdoctoral research fellow to work on the development of sensitive methods of detecting protein sequence homology. A database scanning algorithm will be developed both for the VAX mainframe computer and for supercomputers. There will also be opportunities to use interactive molecular graphics to predict the three-dimensional structure of proteins relevant to cancer.

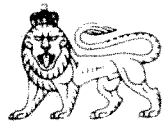
The appointee must be familiar with computer programming and knowledge of protein sequences is desirable. Initial salary will be in the range of £14,000 to £16,000 depending on age and experience.

For further details contact Dr Mike Sternberg on 01-242 0200 ext 2565. Applications, including curriculum vitae and details of three referees, should be sent to Mrs P Harwood, Recruitment Officer, Imperial Cancer Research Fund, 44 Lincolns Inn Fields, London WC2A 3PX, quoting ref 55/R.

Smoking is actively discouraged

I M P E R I A L
CANCER RESEARCH FUND

(8158)A



KING'S COLLEGE LONDON

PROTEIN CHEMIST and MOLECULAR GENETICIST

Applications are invited for two post-doctoral Research Associate appointments, one for three years and the other for one year in the first instance, in Prof H J Gould's group to study the "Receptor Binding Sites on Human Immunoglobulin E", continuing work described in Nature *331*, 180–183, 1988. The protein chemist should have experience of protein purification and binding studies, and an interest in protein engineering. The molecular geneticist should have experience in site-directed mutagenesis.

Inclusive annual salary according to age and experience on a scale £11,315–£17,170.

Further information from Professor Gould on 01–836 8851. Applications with the names of two referees, should be addressed to **Mr D M Drummie, Department of Biophysics, King's College, 26–29 Drury Lane, London WC2B 5RL.** (8155)A

MEDICAL RESEARCH COUNCIL
CYCLOTRON UNIT, HAMMERSMITH HOSPITAL

requires a RESEARCH OFFICER

within the Biology Group of the Clinical Studies section. The work of the group is linked closely with the Unit's clinical programme of Positron Emission Tomography in man and includes the experimental use of tracer radioisotopes to follow dynamic processes of metabolism and neurotransmission in brain and heart. Some experience in pharmacology and/or physiology would be an advantage.

Salary in the range £8,821–£14,055 (pay award pending) depending on age, qualifications and experience.

For application forms and further details please contact:
Miss Jacky Wood, Personnel Officer, MRC Cyclotron Unit, Hammersmith Hospital, Du Cane Road, London W12 0HS. Telephone: 01–740 3162.

Closing date: 2 weeks.

The Medical Research Council is an Equal Opportunity Employer.

(8154)A

NEW ZEALAND
MOLECULAR BIOLOGISTS
DSIR

The Department of Scientific and Industrial Research has an active research programme in plant molecular genetics, applied specifically at present to the improvement of white clover, ryegrass, grain legumes, vegetables and fruit. Research is also carried out in animal gene mapping, leading to transgenic animal development.

A new policy package provided by Government enables expansion of the current research programme. As a result additional scientist and post-doctoral positions have been created in the Biological Industries Group of seven Divisions of DSIR, based at Auckland, Palmerston North and Christchurch.

Applications and enquiries are invited for the following positions:

Scientist, Plant Molecular Biology:
An experienced molecular biologist is sought to direct a research programme into transformation techniques for apple.
(Division of Horticulture and Processing, Auckland).

Scientist, Plant Molecular Biology:
A scientist is needed to investigate transformation, gene isolation and cloning techniques for trees and ornamental crops.
(Plant Physiology Division, Palmerston North).

Scientist, Plant Molecular Biology:
A scientist is required to join a research project in plant insect resistance.
(Plant Physiology Division, Palmerston North).

Scientist, Plant Molecular Biology:
A scientist is required to work on the use of micro-projectile bombardment to introduce genes into pasture plants.
(Grasslands Division, Palmerston North).

Postdoctoral, Plant Molecular Biology:
A postdoctoral scientist is required to join a team working on cloning bacterial genes encoding anti-fungal proteins for transformation into plants.
(Crop Research Division, Christchurch)

Postdoctorals, Plant Molecular Biology:
Two postdoctoral positions are available to work on insect resistance and viral coat protein genes and introduction of these genes into forage legumes.
(Grasslands Division, Palmerston North).

Scientists, Animal Molecular Biology:
Two positions are available to work with an established gene mapping team on animal traits of economic importance.
(Biotechnology Division, Palmerston North).

Scientist, Animal Molecular Biology:
A scientist is sought to work on ruminant embryology, gene transfer into animals monitoring of transgenic offspring.
(Biotechnology Division, Palmerston North).

Scientist, Molecular Biology:
An immunologist is required to work on the production of monoclonal antibodies for the development of immunoassay kits and scale up of monoclonal antibody production.
(Biotechnology Division, Palmerston North).

Scientist, Molecular Biology:
A scientist is required to study the molecular genetics of fruit rotting fungus *Colletotrichum*.
(Plant Diseases Division, Auckland).

Postdoctoral, Molecular Biology:
A microbial geneticist is needed to characterise bacterial regulatory genes with a view to developing novel biosensor system.
(Grasslands Division, Palmerston North).

Scientists, Molecular Biology
Two positions are available for work on gemini viruses, cassava latent virus and chloris-striate mosaic virus to develop these as high copy expression vectors.
(Plant Diseases Division, Auckland).

Scientists, Molecular Biology:
Three scientists are required to work on RNA viruses with a view to developing resistant white clover, peas and lentils.
(Plant Diseases Division, Auckland).

Conditions of employment to these positions are negotiable and may be short term or permanent. Salaries commensurate with qualifications and experience.

Enquiries about the advertised position should be directed to:

Ana Pickering
Resource Development Scientist
DSIR
P.O. Box 1578
Wellington
NEW ZEALAND
Fax: (64) (04) 724 025

(W5764)A

University of Strathclyde

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

LECTURESHIP IN MOLECULAR PHARMACOLOGY

Applications are invited from candidates with a higher degree in pharmacology, biochemistry or a related discipline for a Lectureship in Molecular Pharmacology. In addition to teaching, the successful applicant will be expected to conduct independent research in molecular pharmacology in the field of pulmonary disease/smooth muscle, within the Cardiovascular and Mediators Research Group. Established expertise in molecular/biomedical pharmacology techniques is essential. Salary: £9,260-£19,310 per annum. USS benefits. Application forms and further particulars (4/89) are available from **Staff Office, University of Strathclyde, Glasgow G1 1XQ.** Closing Date for Applications:– 28 February 1989. (8122)A

AFRC INSTITUTE OF PLANT SCIENCE RESEARCH
NITROGEN FIXATION LABORATORY, UNIVERSITY OF SUSSEX, BRIGHTON BN1 9RQ

BIOCHEMIST

A biochemist is required to join a team studying the nature and function of the metal clusters in nitrogenases. The appointment will be for two years in the first instance and is available immediately. The appointee will work directly with Prof. B.E. Smith, Head of the Laboratory, collaborating with other members of the team and possible outside groups as necessary.

The work will involve anaerobic handling techniques and protein isolation and characterisation. Experience in these areas and in either the application of spectroscopic techniques to the study of metalloproteins or site-directed mutagenesis techniques would be an advantage. Candidates should normally have a first or upper second class honours degree in Biochemistry or related subject with at least 2 years' post-graduate experience. Salary scale £10,026-£13,460, non-contributory pension. Send CV to **Mrs J. A. Durrant at the above address.** *The Agricultural & Food Research Council is an Equal Opportunities Employer.* (8121)A

BIRBECK COLLEGE
University of London
DEPARTMENT OF CRYSTALLOGRAPHY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral research assistantship funded by the Muscular Dystrophy Group. The project involves the expression, purification and crystallization of domains of dystrophin, the protein product of the DMD gene, with a view to a full three dimensional structure determination. Experience in protein biochemistry and structural studies would be an advantage. Salary will be within range £11515 – £13800 p.a. depending on age and experience. Post tenable for up to three years.

Applications in duplicate including a full curriculum vitae and names and addresses of two referees should be sent to the **Assistant Secretary (Personnel), Birbeck College, Malet Street, London, WC1E 7HX by 3rd February 1989.**

Further details can be obtained from Dr. J.M. Goodfellow or Professor T.L. Blundell on 01-631 6368. (8137)A

RESEARCH FELLOW IN ENDOCRINOLOGY

DIVISION OF NUTRITION AND ENDOCRINOLOGY

A Postdoctoral Position is available to study the interactions of dietary lipids and hormones in human breast cancer, and investigate the effects of antiestrogens on the synthesis of specific hepatic proteins.

Experience in endocrine assay techniques and/or steroid biochemistry is required.

This appointment is well suited to an individual seeking either a career in clinical laboratory endocrinology or basic endocrine research. Modern, well-equipped research facility located in northern suburb of NYC. Please submit CV to:

Dr. David P. Rose, Division Chief
Division of Nutrition & Endocrinology
AMERICAN HEALTH FOUNDATION
1 Dana Road, Valhalla, NY 10595.

An equal opportunity employer. (NW3149)A

UNIVERSITY OF SURREY
DEPARTMENT OF BIOCHEMISTRY
Research Officer
The Role of Salivary Proteins in Sensory Astringency

Applications are invited for a post of Research Officer, funded by MAFF, CFDRA and a consortium of industrial sponsors, for three years and available immediately. Applicants should have a good degree in Biochemistry, chemistry or an appropriate applied science, and be willing to apply this expertise to a challenging project investigating the role of salivary proteins in sensory astringency. Practical experience of protein separation (HPLC, electrophoresis), ligand binding (dialysis spectroscopy) or immunoassays would be an advantage. The successful applicant will join the Clinical Biochemistry and Nutrition Research Group. Direct supervision will be by Dr M N Clifford (0483 571281 ext 2506), leader of the Nutrition and Food Chemistry Research Team, and there will be regular meetings with staff of CFDRA and industrial sponsors.

Salary will be on the Research and Analogous 1B scale (£8675 — £10460 pa) depending on age, qualifications and experience.

Applications in the form of a curriculum vitae (3 copies) including names and addresses of two referees should be sent to the **Personnel Office (JLG), University of Surrey, Guildford GU2 5HX, quoting reference 799N by 3 February 1989.** (8133)A

University of Edinburgh
DEVELOPMENTAL MOLECULAR BIOLOGIST

Applications are invited for the position of research associate (RA1B scale) to study the role of homeobox genes in the development of the leech. The post is funded by the SERC for a period of 3 years. Recent graduates or students expecting to graduate this year with a good honours degree are encouraged to apply. Experience with recombinant DNA methods is desirable; a prior knowledge of invertebrate development would be useful but not essential. The successful applicant will be based in Edinburgh (D. Price, Psysiology) and will collaborate with Dr. S. Blackshaw at Glasgow University (Cell Biology) and with Dr. D. Weisblat's group at U. C. Berkeley, California. There may be an opportunity to register for a PhD. Salary from £8,675 to £9,865.

Further particulars can be obtained from the Personnel Office; informal enquiries to D. Price are encouraged (031-667-1011 ext. 2357).

Applications including a c.v. and names of two academic referees should be sent to **The Personnel Office, 63 South Bridge, Edinburgh EH1 1LS** by the 28th February.

PLEASE QUOTE REFERENCE NO. 5627 (8140)A

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— the professionals' choice

SYNTHETIC ORGANIC CHEMISTRY

GRADUATE INTO THE WORLD'S NUMBER ONE PHARMACEUTICAL COMPANY

MSD is the world's number one pharmaceutical company. Our policy is to recruit the best scientists available to participate in the discovery of new drugs. We have a number of vacancies for experienced or newly qualified graduates to work on the synthesis of biologically active molecules for the treatment of diseases of the central nervous system. This specialist area calls for talented individuals who can blend academic ability with our pioneering approach. A good grounding in organic chemistry is essential.

Successful applicants will benefit from exceptional facilities at our extensive research centre and the opportunity to work with people renowned worldwide. Those who join us will find real potential to progress their future in research and will participate fully in all aspects of the scientific projects at the centre, which include contributing to published articles. Attendance at external seminars and conferences is positively encouraged.

Salaries are extremely competitive and benefits include: private health care, pension scheme, free life assurance, an active sports and social club and initial housing assistance. An added bonus is our beautiful rural location on the Herts/Essex border, close to major road and rail links and 30 miles from central London.

Please write with full curriculum vitae and salary details where appropriate to Cathy Woodbine, Personnel Manager, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR.

MERCK SHARP DOHME

Neuroscience Research Centre

(8157)A

POPULATION BIOLOGISTS

The Program in Ecology, Evolution, and Behavior of the Department of Biology at Princeton University plans to appoint two population biologists to begin in September 1989, one a tenure-track Assistant Professor, the other at an open rank. Preference will be given to candidates with a strong research program involving field work or theory in ecology and evolutionary biology. The current faculty of Princeton's Graduate Program in Ecology, Evolution, and Behavior are: John Bonner, Jim Gould, Peter Grant, Henry Horn, Steve Hubbell, Marty Kreitman, Bob Lisk, Naomi Pierce, Dan Rubenstein, and Ted Sullivan. The closing date for applications is March 3, 1989. Applicants should send a curriculum vitae and arrange for three letters of reference to be sent to **Dan Rubenstein, Search Committee, Program in Ecology, Evolution, and Behavior, Guyot Hall, Princeton University, Princeton, NJ, 08544-1003.**

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW3092)A

TRUDEAU INSTITUTE, INC.

POSTDOCTORAL ASSOCIATE TO STUDY T CELL-MEDIATED IMMUNITY

A vacancy exists for a postdoctoral fellow interested in studying immunity to tumor and histocompatibility antigens. Current studies deal with various aspects of T cell-mediated rejection of tumors and allografts. The opportunity to study T cell-mediated immunity to bacterial infection also exists. Applicants should have basic training in immunobiology. Starting salary will be commensurate with experience and includes liberal fringe benefits. Subsidized institute housing is available.

The Trudeau Institute is a private, not-for-profit, basic medical research institution located on the shore of Saranac Lake and near Lake Placid, N.Y.

Send curriculum vitae and names of three references to:
Dr. Robert J. North, Director Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983. (NW3080)A

GROUP LEADER POSITION IN MOLECULAR NEUROBIOLOGY

Friedrich Miescher-Institut Basel, Switzerland

The Friedrich Miescher-Institut, a foundation devoted to basic research in cell biology, invites applications for an independent group leader position in neurobiology. Candidates are sought who have an interest in using molecular biology techniques to study the differentiation and the regeneration of the neuronal cell in vitro and in vivo. The candidate should have at least three years postdoctoral experience in molecular aspects of cellular neurobiology. The appointee's group will join two other groups working in the field of neuronal development, led by Dr. Andrew Matus (cytoskeleton) and Dr. Denis Monard (growth factors).

The Institute has strong research programs in cellular and molecular biology and offers an outstanding scientific environment with excellent research facilities. Employment conditions for the offered position are highly competitive. The envisaged starting date is mid-1989 or later. Applications, including an outline of the candidate's proposed research project, should be submitted by February 28, 1989 to **Dr. Denis Monard, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 BASEL, Switzerland.**

(W5748)A

EXPERIENCED CRYSTAL GROWER

A experienced crystal grower (Senior Research Associate) is needed to join an existing group in the Institute for Materials Research (IMR) at McMaster University concentrating initially on the development of techniques for the growth of single crystals of *high temperature oxide superconductors*. The IMR has for many years maintained extensive facilities for preparation, crystal growth and characterization of advanced materials, supported by a technical staff, including a crystal grower, who interact with a number of chemistry, physics and materials science faculty. It is proposed to expand the existing crystal growth capability of the IMR by the addition of one senior crystal grower, together with appropriate equipment and technical support. Ideally, candidates will have a PhD in a relevant discipline such as solid state chemistry: solid state physics or materials science with a minimum of three years experience beyond the PhD or a Master's or Bachelor's degree with eight or more years of experience. Previous experience with a range of crystal growth methods is highly desirable. Salary will be commensurate with the qualifications and experience of the successful candidate.

Applications including a C.V. and a list of at least three professional references should be sent before March 31, 1989 to:

Dr. A. John Berlinsky, Director
Institute for Materials Research
McMaster University
1280 Main Street West
Hamilton Ontario
L8S 4M1

(NW3034)A

Director, Genetic Diagnostic Laboratory:

A position available immediately for a Ph.D. with experience in molecular biology/biochemical genetics to direct a genetic diagnostic laboratory. There is ample opportunity for independent research and faculty appointment at Duke University Medical Center (rank dependent on experience). The candidate will join an established and growing, comprehensive academic genetic and metabolic program with expertise in clinical genetics, molecular and biochemical genetics associated with a major mass spectrometry facility. Duke University is an Equal Opportunity/ Affirmative Action Employer. Send curriculum vitae and three references to:

Y.T. Chen, M.D., Ph.D.
Associate Professor of Pediatrics
Division of Genetics and Metabolism
Department of Pediatrics
P.O. Box 3028
Duke University Medical Center
Durham, North Carolina 27710 (NW3161)A

ASSISTANT OR ASSOCIATE PROFESSOR OF PHYSICAL SCIENCES EDUCATION

Teach courses in one of the departments of the Physical Sciences area, engage in scholarly activities, and assist with development and implementation of a program in Physical Sciences Education. Appointment at rank appropriate to qualifications ($27,588 to $48,204). Applicant must have Ph.D. degree in one of classical physical sciences areas, broad background in the physical sciences and demonstrated emphasis in science education as a primary interest. Applicants are to submit letter of application and curriculum vitae to: **Dr. James D. Crum, Dean, School of Natural Sciences, California State University, San Bernardino, CA 92407**. Applications will be accepted until position filled: will not close prior to 1 February 1989. *An Equal Opportunity, Affirmative Action Section 504, Title IX Employer.* (NW3164)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF VETERINARY PATHOLOGY

RESEARCH ASSOCIATE: POSTGRADUATE/ POSTDOCTORAL

Applications are invited for a 1 year appointment funded by the Agricultural and Food Research Council, with a possible extension, pending grant renewal, for a further 5 years to work in fundamental aspects of the sheep immune system. Candidates should have a Ph.D. or a First Degree in immunology and some research experience, preferably including molecular biology techniques. The work will include characterising sheep IL-2 and the IL-2 receptor with monoclonal antibodies and heterologous cDNA probes. The reagents developed will be used to investigate the immune response in sheep with persistent viral infections. Appointees at postdoctoral level will be paid on the 1A scale, or at postgraduate level on the 1B scale with placement according to age, qualifications and experience.

Informal enquiries may be made to Dr R. Bujdoso, Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH. Telephone 031 667 1011 ext 5245.

Further particulars of this post are available from the **Personnel Office, 63 South Bridge, Edinburgh EH1 1LS**, with whom applications by letter including c.v. and the names and addresses of two referees, should be lodged not later than 9th February 1989. *Please quote reference No. 5624.*

(8040)A

Research Associate

The Department of Clinical Neurological Sciences has a grant funded position available immediately for a Ph.D or M.D. with experience in one or more areas of cortical or cellular physiology, experimental epilepsy or behavioral neuroscience to carry out independent research.

Salary minimum: $21,000. Excellent benefits. Position SO17.

In accordance with Canadian Immigration requirements this advertisement is directed to Canadian citizens and permanent residents of Canada.

Please submit curriculum vitae, list of publications and the names of three referees by February 10, 1989:

Martha Harley
Employee Relations Officer
The University of Western Ontario
Stevenson Lawson Bldg.
Room 262 London, Ontario
N6A 5B8

"An Equal Opportunity Employer"

(NW3157)A

THE AUSTRALIAN NATIONAL UNIVERSIT

THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

Division of Cell Biology

POSTDOCTORAL FELLO
RESEARCH FELLOW
(ORGANIC CHEMIST)

Applications are invited fr persons with a PhD in synth organic chemistry for a positio the level of postdoctoral fell research fellow, depending u research experience, expected be available for a minimum of t years from the first half of 19 Knowledge of carbohydrate che istry and postdoctoral experie is desirable. The appointee will involved in a project concer with the syntheses and modifi tion of sulphated polysacchari and phosphosugars being stud as potential novel anti-inflamn tory and anti-tumor agents.

Applications, including co plete curriculum vitae, brief d cription oif research interests a the names of three referees sho be forwarded in duplicate to t Registrar, The Australian Natio University, GPO Box 4, Canbe ACT 2601, Australia, (quoti reference: JC 5.1.1) by *24 Feb ary 1989*. Enquiries may be dir ted to Dr C. R. Parish, Division Cell Biology, Telephone 49.260

Salary: For a Postdocto Fellow Grade I — will be det mined at a fixed point in the ran A$26,617 to A$30,360. For Research Fellow the salary ran (incremental) is A$30,737 A$40,100.

Appointment at either gra will be limited to two years in ally. Some extension may possible, following review, sho funds be available for this purpos

The University reserves t right not to make an appointme or to appoint by invitation at a time. Further information is av lable from the Registrar, or fr Appointments (35930), Associati of Commonwealth Universiti 36 Gordon Square, Lond WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W5761)A

nature

Still first with the best in science

VIROLOGIST

The Department of Microbiology, North Carolina State University, has available a research and teaching position as Assistant Professor in Virology. A Ph.D. degree with emphasis in plant, bacterial, or animal virology and relevant postdoctoral experience are required. The position is a 12-month tenure-track appointment and includes continuing institutional research support. The successful candidate is expected to teach, direct graduate students and develop a vigorous research program in a basic aspect of virology. Excellent opportunities exist for collaboration with other virologists on campus and in the Research Triangle Area.

Applications are invited from qualified individuals with expertise in virology. Included in the application should be a statement of research interest, curriculum vitae and list of publications. In addition, provide the names, addresses, and telephone numbers of three individuals who may be contacted for a reference by the search committee. Indicate your professional association with each referee. Application materials should be sent to:

Dr. Thoyd Melton, Virology Search Committee, Department of Microbiology, Box 7615, North Carolina State University, Raleigh, NC 27695–7615.

The closing date for applications is February 28, 1989 or until a suitable candidate is selected. Proper documentation of identify and employability will be required before the hiring process can be finalized. *North Carolina State University is an equal opportunity employer.* ((NW2975)A

FACULTY POSITION

HIV VIROLOGIST/MOLECULAR BIOLOGIST
ST. JUDE CHILDREN'S RESEARCH HOSPITAL

The Department of Virology and Molecular Biology at St. Jude Children's Research Hospital has a position available at the junior or senior level for an individual with research interests in the molecular biology and pathogenesis of human immunodeficiency virus (HIV). Candidates should have a Ph.D., M.D., or veterinary degree with research accomplishments that reflect an ability to maintain an independent program. We are seeking to complement our clinical program on AIDS and add to our current research programs which include studies of the replication and pathogenesis of influenza and paramyxoviruses, iridorviruses, adenoviruses and EBV.

The 17-member Department of Virology and Molecular Biology is part of St. Jude's multidisciplinary staff of 120 faculty and 60 postdoctoral fellows engaged in both clinical and basic research. The positions, which are well supported by institutional resources, offer opportunities for collaborative research with members of the Immunology, Tumour Cell Biology, Biochemistry, and Pharmacology Departments, as well as with members of the clinical staff.

The Institution has Core facilities for the synthesis of oligonucleotides and peptides, for peptide and DNA sequencing, a hybriodoma facility, an electron microscope facility, two shared flow cytometers, a molecular modeling/molecular biology computer facility, and a state-of-the-art animal resource center. A new basic research building will provide over 200,000 square feet of laboratory space.

Candidates should send a *curriculum vitae,* including a brief research prospectus, and names of three references to:

Dr. Robert G. Webster, Chairman,
Search Committee, Department of Virology and Molecular Biology,
St. Jude Children's Research Hospital, P.O. Box 318, Memphis, TN 38101–0318.

Equal Opportunity/ Affirmative Action Employer (NW3162)A

California State University
San Bernardino

CHAIR
Department of Biology

Responsibilities: The chair will be expected to provide leadership, encourage excellence and high standards in both teaching and research, facilitate interactions among departmental faculty and with other disciplines, develop and execute a long-range plan for the department, manage the departmental budget while seeking a broad base of support for the department's programs and represent the department within the university community as well as within regional and national organizations.

Deadline for applications: May 1, 1989.

Qualifications: Earned Ph.D., excellent record of teaching, research and extramural funding; a demonstrated commitment to undergraduate and graduate education; evidence of outstanding leadership and administrative abilities.

To apply: Send letter, resume, three letters of recommendation and transcripts to: **Chair, Department of Biology, Chair Recruitment Committee, c/o Dr. James D. Crum, Dean, School of Natural Sciences, California State University, San Bernardino, 5500 University Parkway, San Bernardino, California 92407.**

California State University at San Bernardino is the fastest growing of the 19 California State University campuses, with approximately 10,000 students, 350 full-time faculty, and 50 undergraduate and graduate degrees offered through both daytime and evening programs.

Equal Opportunity, Affirmative Action, Section 504, Title IX Employer.
(NW3165)A

Cambridge NeuroScience Research is a research and development company which focuses on Neurobiology and its applications. We have a new, well-equipped facility located in Cambridge, Massachusetts, close to M.I.T. and Harvard University. We are seeking a Group Leader and Staff Scientist for our Neurotropic Growth Factor Program.

Senior Scientist—Protein Chemistry

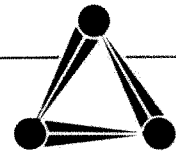
The candidate should have a Ph.D. with a minimum of 3 years' post-doctoral experience with state-of-the-art techniques, including HPLC and other microanalytic methods of protein purification and characterization. The successful applicant will have developed a reputation for independent research.

Staff Scientist—Cell Biology

The candidate should have a Ph.D. and at least three years' experience with the culture of cells from the nervous system.

These scientists will interact with molecular biologists, cell biologists, and neuropharmacologists within the Company and with the Company's extensive network of well-respected neuroscience consultants.

Cambridge NeuroScience Research offers a stimulating research environment with competitive salaries and excellent benefits, including Founders Stock. **Applicants for the above positions should send their curriculum vitae and the names of three references to: CNS Research, Human Resources Manager, One Kendall Square, Building 700, Cambridge, MA 02139, U.S.A.**



CNS RESEARCH

(NW3163)A

SCIENTIFIC DIRECTOR

AVT

A.V. THOMAS GROUP COMPANIES, the South India based major plantation and expanding biotechnology Organization, is seeking a Scientific Director for their Biotech Division. The Division has two seperate research-cum-production facilities with a capacity of producing 6 million plants per annum for export markets. The main facility is located in the Cochin Export Processing Zone and provides top class research facilities for investigation into plant micropropagation, plant health, cell-biology, biochemistry, horticulture and plant breeding. The division also plans to establish an advanced technology training centre for investigation into molecular biology and recombinent DNA technology.

The primary responsibility of the position is the direction of Research Groups covering the areas of Micropropagation, Cell biology, Somatic embryogenesis, Germplasm development, new technology mechanisation, Horticulture and large scale production systems, and supervision of 130 technical personnel.

Other responsibilities would include the constant monitoring of the research interaction and information flow with the sales and marketing agents/departments and production units, the continuation of productive relationships with local universities and government research stations and the maintenance of smooth interface between the biotechnology centre and the company's R & D programme on the field management of plantation crops and quality control unit of the Commodity Exports Department.

Candidate would be required to have a Ph.D in Plant Science with 10 or more years experience in the Plant Science related industries. A broad background in all areas of Plant Science and particularly Plant tissue Culture/Plant Biotechnology would be expected as well as demonstrated management skills.

Salary will be commensurate with qualification and experience and the company is offering an excellent fringe benefit package, free accommodation and car. Please submit a letter of application, curriculum vitae, statement of current research activities and the name and address of 3 references within 10 days of this advertisement. to:

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ADVANCED BIOCENTER
2 LANCASTER HOUSE
COX'S YARD
SOMERTON, SOMERSET
UNITED KINGDOM
TEL: 0458 74073
TLX: 94012204 ABIO IN

(8144)A

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PATHOLOGY

A GRADUATE RESEARCH POSITION is available to work on a Medical Research Council funded project concerning the use of cultured skin keratinocytes as allografts. The post which is for twelve months is available from 1 February 1989.

Applicants should have experience in immunohistochemical and ultrastructural techniques.

Applications in writing with c.v. and names and addresses of two referees to **The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. PLEASE QUOTE 9114**

Further details can be obtained from Dr. Margaret Stanley, Telephone (0223) 333736. (8151)A

AFRC

AFRC INSTITUTE OF ARABLE CROPS RESEARCH ROTHAMSTED EXPERIMENTAL STATION HARPENDEN HERTS AL5 2JQ

PROTEIN CHEMIST

required to initiate and collaborate in studies of the structures and reaction mechanisms of plant enzymes with key roles in metabolism. It will include ongoing studies of the structure and active site chemistry of Ribulose Bisphosphate Carboxylase/Oxygenase (RuBisCO). The person appointed will be responsible for the design of mutant and chimaeric forms of the enzymes, and for analysis of their structures and catalytic properties. Ref. 779.

Qualifications: First or Upper Second Class Honours degree in Biochemistry, chemistry or a related subject, with at least four years postgraduate experience.

Appointment in the grade of Senior Scientific Office on scale £12,445-£17,032. Non-contributory superannuation.

MOLECULAR BIOLOGIST

required to join a group studying the structure and expression of cereal seed genes. The person appointed will collaborate in studies of cereal seed development, and the structure and control of expression of genes for major seed proteins. The work will involve construction and characterization of cDNA libraries, isolation of genomic clones, DNA sequencing and possibly *in situ* hybridization of cDNAs. Previous research experience and an interest in plant development will be an advantage. Ref. 778.

Qualifications: First or Upper Second Class Honours degree in Biochemistry, Molecular Biology or a related subject with at least two years relevant post-qualifying experience.

Appointment in the grade of Higher Scientific Officer on scale £10,026-£13,460. Non-contributory superannuation.

Applications with full cv and the names of two referees should be sent to **The Secretary** quoting the appropriate Ref. No. by 10 February.
We are an equal opportunities employer (8161)A

UNIVERSITY OF LEICESTER DEPARTMENT OF MICROBIOLOGY POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a Research Associate post to work on development of new reagents for the diagnosis of tuberculosis. This post, which is funded by the World Health Organisation, is available immediately for one year. However, in line with WHO requirements, an application for renewal of funding will be made after one year. Salary (according to age and experience) on the scale IA: £9,865–£15,720.

Applications (including c.v. and the names of two referees) should be sent to either **Dr. P.W. Andrew or Dr. G.J. Boulnois, Department of Microbiology, P.O. Box 138, Medical Science Building, University Road, Leicester, LE1 9HN.** For informal enquiries call Dr. Boulnois on 0533–522944. (8037)A

POSTDOCTORAL POSITIONS MOLECULAR BIOLOGY, VIROLOGY, IMMUNOLOGY

Postdoctoral positions (3) are available for persons to participate in multidisciplinary experimental studies of Simian Immunodeficiency Virus expression relative to: (1) mechanisms of pathogenesis and development of effective immunoprophlaxis; (2) evaluation of antiviral drugs in vitro and in vivo; and (3) identification of humoral and cell-mediated immune responses to SIV infection. Please send curriculum vitae, a summary of research interests, and the names of three references to **Drs. Michael Murphey-Corb, Kenneth Soike,** or **Louis Martin,** respectively, **Delta Regional Primate Research Center, Three Rivers Road, Covington, LA. 70433.** (NW3160)A

POSTDOCTORAL POSITION

Cellular Immunologist/ Molecular Biologist

Postdoctoral position in mucosal immunology to study immunmechanisms in the gastrointestinal tract and their relationship to endocrine function. Preference will be given to candidates with training in cellular immunology and/or molecular biology. Salary is paid according to BAT IIa/BAT III amounting to approx. $33,000 per annum. Send curriculum vitae and names of three references to: **Dr. Rudolf Hatz, Department of Surgery, Klinikum Grosshadern, Ludwig Maximilians University of Munich, Marchioninistr, 15, 8000 Munich 70, Federal Republic of Germany.**

(W5768)A

AFRC Institute of Food Research Reading Laboratory

Higher Scientific Officer/Senior Scientific Officer

A Physical chemist is required for a new project on the crystallisation of fats used in food manufacture. Functional properties of triglyceride mixtures will be studied in both bulk phase and emulsion systems. Technical support will be provided.

Qualifications: Honours degree (I or IIi) in Chemistry or related discipline, preferably with a PhD. Experience with x-ray diffraction, especially AND, DSC and NMR would be advantageous. At least two years relevant experience is required for HSO and at least 4 years for SSO.

Salary £10,026-£13,460 — HSO £12,445-£17,032 — SSO.

Please write for an application form to the Personnel Officer, AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT.

Please quote reference (46/88). Closing date: 6th February 1989.

The AFRC is an equal opportunities employer. (8163)A

THE KENNEDY INSTITUTE OF RHEUMATOLOGY CELL ENZYMOLOLGY UNIT

A recently qualified graduate is required to join a small and enthusiastic group working on inherited disorders of lysosomal function. The scientist will be studying lysosomal enzymes in different types of lymphocyte and lymphold cell, using a variety of contemporary biochemical and immunological techniques. Interest in basic aspects of cell biology would be an advantage.

The appointment is for 18 months with the possibility of renewal thereafter. The starting salary is up to £10,710 inclusive of London Weighting. Applications including a c.v. and the names of two referees should be sent to The General Secretary, Kennedy Institute of Rheumatology, 6 Bute Gardens, Hammersmith, London W6 7DW by 9 February 1989.

(8125)A

AFRC Institute of Food Research Reading Laboratory

Scientific Officer/Higher Scientific Officer

A Food materials scientist is required for a 2 year EEC-funded project assessing Mozzarella cheese functionality by sensory and instrumental means. The work will require collaboration with scientists in other specialisms and with industrial technologists.

Qualifications: Degree in Food Science, Food Technology or a related subject or HNC with relevant experience. At least 2 years' experience is required for appointment to the higher grade.

Salary: £8,574-£10,994 — SO £10,026-£13,460 — HSO.

Please write for an application form to the Personnel Officer, AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT.

Please quote reference: 52/88. Closing date 6th February 1989.

The AFRC is an equal opportunities employer. (8162)A

ACADEMIC PHYSIOLOGIST

The Department of Anesthesiology of Loyola University Stritch School of Medicine, Maywood, Illinois is recruiting a physiologist for a faculty position at the Assistant Professor level to work in a stimulating environment that includes excellent research laboratory. The candidates for this position are expected to have a strong commitment to innovative research in cardiovascular and respiratory physiology and ability to run large animal experiments in collaboration with the departments physician faculty and resident trainees. The salary is competitive and Loyola University is an Equal Opportunity Employer.

Please submit curriculum vitae, reprints, a statement of research interests, and arrange to have three letters of recommendations sent directly to:

Tadikonda L.K. Rao, M.D
Department of Anesthesiology
Loyola University
2160 S. First Avenue
Maywood, Illinois 60153 (NW2895)A

MOLECULAR VIROLOGIST

A staff position is available for a person with experience in the molecular biology of primate viruses. The successful candidate would be expected to become involved with research programs involving simian immunodeficiency disease as well as to establish his/her own area of research. Ongoing research includes studies investigating the interrelationships of opportunistic viruses with Simian Immunodeficiency Virus. The applicant would be expected to obtain research support as well as to work collaboratively with other investigators. Please send résumé, a summary of research interests, and the names of three references by Feb. 15 to: **Dr. Michael Murphey-Corb, Delta Regional Primate Research Center, Three Rivers Road, Covington, LA 70433.** (NW3159)A

LECTURER – VERTEBRATE AND FIELD BIOLOGY

The Program in Ecology, Evolution, and Behavior of the Department of Biology at Princeton University has a full-time opening or two half-time openings at the rank of Lecturer for a one year renewable appointment beginning in September, 1989. Duties include teaching a Vertebrate Biology Laboratory course with a strong physiological component, teaching a Field Biology course, and advising the independent work of several undergraduates in these fields. The courses are given during the Fall term, in collaboration respectively with Robert Lisk and Henry Horn, and there is time in the Spring term for the candidate's own research. A PhD in a relevant discipline is required. Applicants should specify whether they wish a half-time or full-time appointment. The closing date for applications is March 3, 1989. Applicants should send a curriculum vitae and arrange for three letters of reference to be sent to **Robert Lisk, Search Committee, Program in Ecology, Evolution, and Behavior, Guyot Hall, Princeton University, Princeton, NJ, 08544-1003.**

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW3091)A

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NATIONAL KIDNEY RESEARCH FUND

RESEARCH FELLOWSHIPS

The National Kidney Research Fund (NKRF) is to offer two Fellowships, one clinical and one non-clinical in research areas related to renal disease. The Fellowship may be held in any suitable department in the UK. Fellows will be appointed for three years in the first instance, but an extension for a further two years will be possible. A grant of £5,000 per annum for research expenses will be available to the host department. The clinical Fellowships salary will be between £13,470 and £19,610 depending on experience. The non-clinical Fellowship salary will be at the appropriate post-doctoral level.

Closing date for receipt of applications 6th March 1989.

Application forms may be obtained from:

The Director-General, NKRF, 42 Lower Marsh, London SE1 7RG. (8099)E

THE ROYAL VETERINARY COLLEGE
University of London

POST-DOCTORAL RESEARCH FELLOWSHIP IN MOLECULAR BIOLOGY

LATENCY OF EQUID HERPESVIRUS I

Applicants will be invited to participate in an existing project investigating the role of latency, particularly of the immediate early proteins in the control of EHVI. Further information is available from Dr N Edington. Technical help will be available for three years.

SALARY according to age and experience will on the Universities Research Scale 1A, not less than £11,515 per annum, including London Allowance. Eligibility for Universities' Superannuation Scheme.

Applications with a cv and the names of two referees should be sent to the **Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU.** (8136)E

Prize Postdoctoral Fellowship

The Center for Photochemistry and Photobiology at the University of Chicago invites applications for the James Franck Postdoctoral Fellowship for 1989. The James Franck Fellow will work on an interdisciplinary project dealing with photochemical reaction centers for bacteria, cyanobacteria, green algae or plants using a combination of the techniques of molecular genetics, biochemistry, absorption and magnetic resonance spectroscopy, X-ray crystallography, and ultrafast fluorescence spectroscopy. Faculty supervisors include Professors G. Closs, G. Fleming, M. Garavito, R. Haselkorn, G. Lamppa, L. Mets and J. Norris. The fellowship carries a 12-month stipend of $32,000. There are no restrictions on age, citizenship or length of time since the Ph.D. was earned. Please address inquiries to **R. Haselkorn, Director of the Center, 920 E. 58th Street, Chicago, IL 60637.** (NW3150)E

SCHOOL OF PHARMACY
UNIVERSITY OF LONDON

TOXICOLOGY UNIT

APPLICATIONS ARE INVITED for the above post available immediately for a fixed term of three years. This project involves investigations to understand basic mechanisms of chemical induced toxicity. The study will concentrate on the roles of pyridine nucleotide depletion and poly ADP-ribosylation in cellular toxicity.

Applicants should have a good honours degree in Biochemistry or a related subject. Candidates with a Ph.D may be appointed as a Research Fellow. Salary on RA 1B scale for Research Assistants (£8,675-£11,680 + £1,650 London Weighting) or RA 1A for Research Fellows (£9,865-£12,120 + £1,650 L.W.) Informal enquiries to Dr G M Cohen (01-837-7651 Extn 71).

Applications in the form of a CV including names and addresses of two referees to: **The Assistant Secretary, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX.**

Closing date for applications: 3 weeks after publication date. (8113)E

University of York
Department of Biology
CANCER RESEARCH UNIT

Post-doctoral Positions

Two positions are available in the above Unit which specialises in cancer aetiology relating to chemicals.

Position 1 is a one-year fellowship with the possibility of extension up to a maximum of 3 years in total, to investigate the role of oncogene activation and chromosome alterations in human colon cancer, using tissue culture techniques.

Position 2 is a two-year fellowship funded by the European Commission as part of the collaborative programme on 'Biomonitoring of human populations exposed to genotoxic environmental chemicals'.

Applicants for both posts should have, or expect to gain shortly, a PhD in a relevant topic.

Salary for both positions will be in the range 1A for Research and Analogous Staff (£9,865-£15,720) per annum.

Informal enquiries to Dr Colin Garner on 0904 432903.

Four copies of applications, with full curriculum vitae and naming two referees should be sent by Monday 6 February 1989 to Personnel Office, University of York, Heslington, YORK, YO1 5DD, from whom further particulars are available. Please quote advertisement reference number 7/6277. (8106)E

UNIVERSITY OF BIRMINGHAM
School of Physics & Space Research

Research Fellowship (Ref PH 5399)

STUDY THE SUN WITH THE SOLAR PHYSICS GROUP

We have stations in Hawaii, Western Australia and Tenerife and have a major SERC award to expand and develop this network. The globa oscillations of the Sun studied by the group enable one to probe the depths of the Sun and to study its physics to a precision rare ir Astronomy. Applications are invited from PhD level candidates ir Physics, Astronomy or Control Engineering for a person who car contribute substantially to the development and implementation o the stations, the observing programme or the data interpretation Prospective applicants may wish to contact Professor G Isaak, Schoo of Physics and Space Research (tel 021-414 4575), for an informa discussion of this exciting research opportunity. It is expected that th appointment will be within the first three points of the Researcl Fellow 1A scale, £9,865–£11,070 plus superannuation.

For further particulars of the above post and an application form, tele phone 021-414 6383 quoting reference number PH 5399. Application (3 copies) should be sent to the **Senior Assistant Registrar, Faculty o Science, PO Box 363, Birmingham B15 2TT** by 10 February 1989.

AN EQUAL OPPORTUNITIES EMPLOYER

(8102)E

Applications are invited for the

M.C. Chang Postdoctoral Fellowships

in

Male Fertility Research

supported by the Andrew W. Mellon Foundation at

The Worcester Foundation for Experimental Biology

The Worcester Foundation's Male Fertility Program emphasizes multidisciplinary approaches to the study of male reproductive biology utilizing state-of-the-art techniques in biophysics, biochemistry, and cell and molecular biology. The faculty and their research interests are:

Lawrence J. Dangott: Receptors and mechanisms of chemical signaling in spermatozoa

Grant Fairbanks: Mechanism of sperm plasma membrane modification during epididymal maturation

Daniel L. Kilpatrick: Gene transcription during spermatogenesis using transgenic mice; paracrine function of testicular peptides

John A. McCracken: Studies on sperm maturation, motility, and capacitation using surgical diversion of the male and female ducts

Clare M. O'Connor: Regulation of protein modification and turnover during gametogenesis

George B. Witman: Control of sperm motility during maturation and capacitation; phosphorylation of sperm flagellar proteins

David E. Wolf: Diffusion and control of membrane regionalization, sperm maturation, capacitation, and fertilization

Carol A. Ziomek: Membrane structure and cell polarity in early mouse development

The Worcester Foundation is a private, nonprofit biomedical research institute offering excellent facilities and a stimulating research environment. Please send curriculum vitae and names of three references to the faculty member of interest, c/o **Male Fertility Program, Personnel Office, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.**

WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY

AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER M/F/H

(NW3147)E

NIH FUNDED FELLOWSHIPS

r post-doctoral study on varilla-zoster virus latency at the iversity of Colorado School of edicine. Apply to: **Dr. Donald lden, Dept. of Neurology, Unirsity of Colorado School of edicine, 4200 E. 9th Ave., enver. CO 80262.** AA/EOE.

(NW3158)E

GULBENKIAN INSTITUTE OF SCIENCE

Oeiras, Portugal

GULBENKIAN RESEARCH FELLOWSHIPS IN BIOLOGY

The Department of Science of the Calouste Gulbenkian Foundation is offering two research fellowships for postdoctoral scientists wanting to work at the Gulbenkian Institute of Science (Biology), Oerias, Portugal. Candidates should be able to work in one of the following areas: Molecular biology of eukariotic cells; Molecular and general virology (African Swine Fever Virus); Neuro-biology of purines.

The fellowships are for two years. The stipend is 180 000 portuguese escudos per month. Travel assistance may be provided.

Applications should be submitted in duplicate to the **Director, Gulbenkian Institute of Science, Apartado 14, 2781 Oeiras Codex, Portugal.** They should include a curriculum vitae, the area of research selected from one of those indicated above, and the names and addresses of two referees. The closing date is two weeks from date of publication.

(W5767)E

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BP

BP Research Fellowships

The Royal Society of Edinburgh invites applications for these prestigious 3 year post-doctoral Research Fellowships, to be held in Scottish Universities and Central Institutions.

Fellowships will be available for independent research in:

Mechanical Engineering	Solid State Sciences
Information Technology	Organic Chemistry (except natural product and biological chemistry)
Geological Sciences	

Applicants must have a Ph.D. and be able to demonstrate an excellent record of innovative research. Preferred age range 28–35. Salaries will be on University Research grades 1A and 2 (in the range £12,150 to £17,630) with placement according to experience.

Application forms are available from **The Executive Secretary, The Royal Society of Edinburgh, 22–24 George Street, Edinburgh EH2 2PQ,** and must be returned by 1st March 1989.

(8101)E

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1989 AWARDS

Next deadline: 10 February 1989

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are usually awarded initially for one year, but with the possibility of renewal for a second year. In cases of exceptional scientific merit, however, the selection committee can award a 2-year fellowship. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 10 February 1989. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 17 April 1989.

Application forms and further details may be obtained from **Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.** Inquiries should be accompanied by a self-addressed adhesive label. (W5559)E

STUDENTSHIPS

NATIONAL KIDNEY RESEARCH FUND

STUDENTSHIPS

The National Kidney Research Fund (NKRF) is to offer three Studentships in research areas related to renal disease. Heads of department are invited to apply for studentship allocations. Student stipends vary between £3,690 p.a. (outer London) £4,500 (London) plus approved student fees and contribution to departmental research expenses.

Closing date for receipt of application 6th March 1989.

Application forms may be obtained from:

The Director-General, NKRF, 42 Lower Marsh, London SE1 7RG. (8100)F

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

DEPARTMENT OF SURGERY

NUTRITIONAL CONSULTATIVE PANEL SCHOLARSHIP

Applications are invited for a three year research studentship into "The Effect of Dietary Calcium on the Development of Human Colorectal Cancer". The successful applicant will be registered for a higher degree. The studentship is funded by the Nutritional Consultative Panel and the terms and conditions are similar to those for an MRC studentship.

Candidates should possess a good honours degree in biochemistry, pharmacology or related discipline. The project will be supervised by Professor R C N Williamson, M.A., M.D., M.Chir, F.R.C.S., Professor and Director of Surgery, Royal Postgraduate Medical School.

Applications together with a Curriculum Vitae and the name and addresses of two referees should be sent as soon as possible to **Mr P W Davies, Departmental Superintendent and Assistant General Manager (Surgery), Department of Surgery, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN.** (8043)F

KING'S COLLEGE LONDON BIOMEDICAL SCIENCES DIVISION

POSTDOCTORAL FELLOWSHIP

Applications are invited for a postdoctoral fellowship (3 years) in an active research group, studying the regulation and mechanism of insulin secretion.

Applications should preferably have training or research experience in molecular biological techniques applicable to the study of secretory processes.

Starting salary on Research Fellow 1A Scale in range £11,515 – £13,800 per annum (including London Allowance).

Applications, including Curriculum Vitae and the names of two referees, to:

Professor S L Howell, Biomedical Sciences Division, King's College London, Campden Hill Road, Kensington, London, W8 7AH. (8114)E

FRIEDRICH MIESCHER INSTITUT BASEL, SWITZERLAND

POSTDOCTORAL FELLOWSHIP

Applicants are invited for an internally funded fellowship of up to 3 years maximum duration to join an established team working on the role of methylation in eucaryotic gene regulation and on the interactions of transacting factors (*in vitro* and *in vivo*) with regualtory elements of hormonally controlled genes. Emphasis will be made on the tissue specific expression of genes. The Institute provides outstanding facilities in a purely research environment.

Position is available in the fall 1989. Candidates should have a good background in cell biology, molecular biology or biochemistry.

Applications (with C.V.) should be sent to Dr J.P. Jost, Friedrich Miescher Institut, P.O. Box 2543. CH 4002 Basel, Switzerland. (W5739)E

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMICAL AND PROCESS ENGINEERING

Research Studentship

Applications are invited from newly qualified Engineers (Chemical or Mechanical) or Scientists (Physics or Materials Science) to work on a study of impact attrition of particulate solids. The project is funded by a grant from the International Fine Particle Research Institute, and aims to relate the attrition and breakdown of particulate solids to their fundamental physical and structural properties and the impact process conditions. Practical aspects concern preparation of test materials, characterisation of flaws and of impact damage. Theoretical aspects involve analysis of stress field, and of formation and propagation of cracks by the use of fracture mechanics principles. The work is of multi-disciplinary nature, and requires a student with flair and initiative. The projects is for a duration of three years leading to a PhD Degree. The University fees and student maintenance are covered by the grant. In the absence of any suitable British applicants, candidates from other nationalities could be considered. Some financial assistance is available for travel to England.

Further details may be obtained from Dr M Ghadiri, tel 0483-509270. Applications in the form of a curriculum vitae including the names and addresses fo two referees should be sent to **Dr M Ghadiri, Department of Chemical & Process Engineering, University of Surrey, Guildford, Surrey GU2 5XH, England. Ref N.** (8128)F

BRITISH MUSEUM (NATURAL HISTORY)

POSTGRADUATE RESEARCH STUDENTSHIPS

Two studentships will be available, tenable for 3 years at the British Museum (Natural History) commencing October 1989.

Applications are invited for the following five research projects, the two to receive studentships will be selected following interviews in March.

Arrangements will be made with an appropriate university for joint supervision of studentships and registration for a higher degree.

Department of Zoology
Taxonomy and biology of fish lice (Crustacea: Branchiura).

Department of Entomology
Molecular evolution related to chromosome evolution in anopheline mosquitoes.

Department of Palaeontology
British Silurian Bryozoa.

Department of Botany
Scanning Electron Microscope investigation of the structure, ornamentation and ontogeny of the oosporangial wall in selected Charalean taxa.

Department of Mineralogy
Structure and composition of boron minerals by soft X-ray spectroscopy and related techniques.

A wide range of scientific facilities is available and students will have full access to the Museum's extensive libraries.

Eligibility
Applicants must be ordinarily resident in Great Britain, and have a first or second class honours degree; a student graduating in 1989 may be offered an award subject to his/her degree results reaching the required standard.

Application forms, conditions of the awards and further information on projects may be obtained from the **Assistant to the Director, British Museum (Natural History), Cromwell Road, London SW7 5BD.**

Closing date for receipt of completed forms is 17 February, 1989.

(8104)F

COURSES & CONFERENCES

UNIVERSITY COLLEGE LONDON

Science and Engineering Research Council Centre for Biochemical Engineering

Biochemical Engineering Postgraduate/Postexperience Taught Course Studies

UCL is a world leader in postgraduate/postexperience teaching of biochemical engineering for chemical engineers, bioscientists and others with appropriate training. The Master's Degree course is suitable for recent graduates. It is used also by companies for post-experience training and complements an annual one week intensive course. Those completing the MSc are strongly sought after by industry.

UCL has an international reputation in the field of biochemical engineering research and has received special government support so that it is superbly well equipped both at laboratory and pilot plant levels. The calendar year MSc course starts in October and makes extensive use of these research facilities to complement biochemical engineering lectures and studies of process engineering or biological science according to background. Biological scientists undertake a three month process design project, while chemical engineers complete a research project alongside the multidisciplinary research team.

Details from Miss Valerie Potter, Department of Chemical and Biochemical Engineering, University College London, Torrington Place, London, WC1E 7JE, UK. (8110)C

INTERNATIONAL CONFERENCE

ATRIAL PEPTIDES AND THEIR NOVEL ANALOGUES
A rational approach to cardiovascular diseases?

15th/16th March 1989, Royal College of Physicians, London

This two-day international conference, organised by IBC Technical Services, will provide a comprehensive update on the progress being made in research on atrial natriuretic factor. Although the factor was identified only six years ago, it has already been purified and sequenced, and a number of congeners, analogues and even antagonists are under development. Native forms of ANF are in clinical trials, and at least one marketing application is in the pipeline. Potential indications include congestive heart failure, hypertension, renal and hepatic disease and asthma.

Chairmen:

Professor G. Burnstock
University College London, UK

Dr M. Cantin
Clinical Research Institute Montreal, Canada

Speakers to include

Dr J.V. Anderson
St Bartholomew's Hospital Medical School, UK

Dr J.M. Berman
Glaxo Research Laboratories USA

Dr E.H. Blaine
G.D. Searle R+D Washington University, USA

Dr C. Brink
CNRS France

Dr M.J. Brown
Addenbrooke's Hospital Cambridge, UK

Dr J. Burnett
Mayo Medical School, USA

Dr. T. Hedner
Sahlgren's University Hospital Sweden

Dr E.J. Johns
The University of Birmingham, UK

Dr G.A. MacGregor
Charing Cross and Westminster Medical School, UK

Dr P. Needleman
Washington University School of Medicine, USA

Professor J.M. Polak
Royal Postgraduate Medical School, Hammersmith Hospital, UK

Dr C.E. Seidman
Brigham and Women's Hospital, Boston, USA

Dr J.D.H. Slater
Middlesex Hospital, UK

Dr P.G. Withrington
Medical College of St Bartholomew's Hospital, UK

Full details from:
Renata Duke,
IBC Technical Services Ltd,
Bath House (3rd Floor),
56 Holborn Viaduct, London EC1A 2EX
Tel: 01-236-4080. Telex: 888870 IBC G (8143)C

Advances in Separations in

Biochemistry Cell Biology and Biotechnology

6th International Conference on Partitioning in Aqueous Two-Phase Systems

Assmannshausen/Rhein
Aug. 27–Sept. 01, 1989

Deadline for abstracts: April 15th, 1989
Registration deadline: May 15th, 1989

Scientific Programme

— Theory and Analysis
— Partitioning of Biopolymers:
Separations of proteins, nucleic acids etc. by extraction, CCD and chromatographic techniques, application of affinity agents
— Partitioning of cells and subcellular organelles (including immuno affinity techniques)
— New Methodology and Emerging Technology (including new phase systems and polymer derivatives)
— Biotechnological Applications: downstream processing, extractive bioconversions and liquid-liquid extractions

Local Organizers
W. Müller, FRG
M.-R. Kula, FRG

Address for Correspondence
All mail including registration, abstract forms should be sent to:
Deutsches Reisebüro GmbH
DER-Congress
Att. Mrs. Brigitte Schönfeldt
P.O. Box 100701
D-6000 Frankfurt, West Germany

(W5769)C

Royal Postgraduate Medical School

(University of London)

Advances in the applications of monoclonal antibodies in clinical oncology

21ST-23RD JUNE 1989

This 6th International Meeting will highlight advances in both fundamental aspects of monoclonal antibodies and applications in diagnosis and therapy of malignant disease. The meeting will be of interest to laboratory scientists, clinicians and members of the biotechnology and pharmaceutical industry.

Topics include:
Humoral and cellular immune control of tumour growth • The importance of antibody dilution • Transport of antibodies into tumours and studies on the internalisation • Tumour site activation of cytotoxic agents • Antibody engineering • Clinical trials with hybrid and chimeric antibodies • Tumour glycoproteins • Biocompatibility and cellular processing of immunoconjugates • Macrocycle chemistry • Technetium labelling of antibodies • Studies of antigen binding sites • Idiotypic antibodies as vaccines • Systemic and reigonal antibody therapy • Radiodosimetry

Speakers and Chairpersons include:
Dr D Allen, Prof K D Bagshawe, Dr R Begent, Dr Benveniste, Dr L Berneman, Dr P Beverley, Sir Walter Bodmer, Dr K Bosslet, Dr R Bradwell, Dr M Clark, Dr N Courtenay-Luck, Dr A Dagleish, Sir Colin Dollery, Dr R Duncan, Prof D Evans, Dr A A Epenetos, Dr C Foster, Mrs V Hird, Dr R K Jain, Prof P Kenemans, Dr G Winter, Prof A F LoBuglio, Dr S Mather, Dr S Matzku, Prof C Meares, Dr M Ritter, Dr J Rothbard, Prof K Sikora, Dr J Taylor-Papadimitriou, Dr B Wessels.

Course Organiser:
Dr A A Epenetos, Senior Lecturer and Constultant in Clinical Oncology, RPMS, Hammersmith Hospital and Imperial Cancer Research Fund, UK

Course fee: £245

Course enquiries: Dr A A Epenetos (tel: 01–740 3149)
Two half days will be open for paper presentations. Abstracts will be considered for oral and/or poster presentation. Manuscripts will be considered for publication by the International Journal of Cancer as a Supplement Issue devoted to this meeting.

Closing date: for registration and submission of abstracts 1st May 1989
for manuscripts 21 June 1989

Application forms, abstract forms and further details
may be obtained from: School Office (SSC), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN
Tel: 01–743 2030 ext 2242 (8119)C

TECANICAL COURSE
ON
HPLC

29th to 31st March 1989

Organised by Harish Patel and Alan D.B. Malcolm
at
Department of Biochemistry
Charing Cross and Westminster Medical School
Fulham Palace Road, London W6 8RF

The course consists of lectures and practical demonstrations.

Registration fee, £95, for participants from non-profit making institutes and £125 for commercial laboratories.

For an application form and provisional programme contact Linda Readings at the above address (01-741 1858).

Closing date for registration: 10th March 1989. (8159)C

UNIVERSITY OF ABERDEEN

M.Sc. in Clinical Pharmacology

Applications are invited for a 12 month full-time post graduate course leading to the degree of M.Sc. in Clinica Pharmacology. The course consists of lectures and practi cal work, in addition to an original research project. The course is designed for medical practitioners or non-medi cal graduates with appropriate qualifications in Pharmacy or Pharmacology or a related subject.

Further details may be obtained from the **Secretary Clinical Pharmacology Unit, Department of Medicine & Therapeutics, University of Aberdeen, Foresterhill Aberdeen AB9 2ZB, to whom application for admission to the course should be made.** (8138)C

COURSE ON SEPARATION TECHNIQUES IN PROTEIN PURIFICATION

Organised by Harish Patel and Alan D.B. Malcolm
Department of Biochemistry
Charing Cross and Westminster Medical School
Fulham Palace Road, London W6 8RF
In conjunction with
PHARMACIA LTD
20th to 22nd March 1989

The course is designed to give practical training in separation techniques.

Registration fee (with lunch and coffee): £95, for participants from non-profit making institutes and £125 for commercial laboratories.

For an application form and provisional programme contact Linda Readings at the above address (01-741 1858).

Closing date for registration: 6th March 1989. (8160)C

ASSISTANTSHIPS

Imperial College of Science, Technology & Medicine

Positions in Molecular Parasitology Postdoctoral Scientist and Graduate Research Assistant

Applications are invited for a Postdoctoral scientific position and a graduate Research Assistantship studying the immunology and molecular biology of nematode parasite infections. Each position is funded for up to 3 years, within the Parasitology research group in the Department of Pure and Applied Biology at South Kensington.

The Postdoctoral project, under the supervision of Dr. R.M. Maizels, centres around identifying key molecular interactions between nematode parasites and their mammalian hosts with a particular focus on direct and indirect (interleukin-mediated) stimulation of host cell subpopulations. The graduate Research Assistant position will be supervised by Dr. J.E. Bradley and is funded for 3 years by the MRC. The project will involve the molecular characterisation of diagnostic antigens of the medically important parasite *Onchocerca volvulus*. Other interests related to these projects are the antigenic nature of the nematode surface, enzymes and other molecules secreted by parasites, interactions with the host immume network, and the epitopic structure of immunologically important nematode antigens.

Applicants with interests in these areas and/or experience with relevant immunological, molecular biological or biochemical techniques should apply, enclosing a C.V. and names of two referees, to the appropriate supervisor at the **Department of Pure and Applied Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB,** by 28 February 1989. (8149)P

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GROWTH AND DIFFERENTIATING FACTORS IN HAEMATOLOGY
P. REIZENSTEIN, L. SACHS

1st-5th May
BMT IN LYMPHOMAS
In collaboration with the European School of Oncology
F. CAVALLI

22nd-26th May
ALLOGENEIC BONE MARROW TRANSPLANTATION
E. GLUCKMAN, T. GORDON SMITH, B. SPECK

28th-29th June
ACUTE AND LATE EFFECTS OF IRRADIATION ON THE HAEMOPOIETIC SYSTEM
Satellite Symposium of the 17th International Congress of Radiology
M. TUBIANA, C. PARMENTIER

2nd-6th October
FIBRINOLYSIS
F. BACHMANN, M. SAMAMA

6th-10th November
DREPANOCYTOSE ET β THALASSEMIE
Session Spéciale en Français
Y. BEUZARD

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GENOME MAPPING AND SEQUENCING

April 26 - 30, 1989

Organized by:

Charles Cantor, *Columbia University*
Maynard Olson, *Washington University*
Richard Roberts, *Cold Spring Harbor Laboratory*

The meeting will focus on:

Ongoing mapping endeavors
New methodologies for mapping and sequencing
Techniques for the analysis of maps and sequences

The organizers invite submission of abstracts concerning recent work for oral or poster presentations by **February 16, 1989.** Requests for registration materials should be forwarded to:

Meetings Coordinator
Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724
516-367-8346 (NW3151)M

IMPERIAL CANCER RESEARCH FUND DNA TUMOUR VIRUS MEETING 1989

The Imperial Cancer Research Fund is sponsoring a meeting on DNA Tumour Viruses to be held in Churchill College, Cambridge, England from *24th to 29th July, 1989* (5 days). The subject of meeting will be the molecular biology of small DNA tumour viruses, particularly polyoma virus, SV40 and adenoviruses. It will replace for this year the annual Cold Spring Harbour DNA tumour virus meeting.

The meeting is open to all interested research workers. The scientific programme will include oral presentations and posters. The cost for registration, accommodation, food and the abstract book will be £260.

Anyone interested in attending the meeting should apply immediately for further details to: **Mrs. Clare Middlemiss, ICRF DNA Tumour Virus Meeting Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England.** (8124)M

WORKSHOP

CHROMOSOME 21: IMPACT OF THE NEW GENOME TECHNOLOGY IN HUMAN GENETICS

Santa Margherita Ligure Genoa (Italy) May 18-20, 1989

SPONSORED BY: The European Molecular Biology Organization The International School of Pediatric Sciences
ORGANIZERS: Nicoletta Sacchi (Frederick, USA); Paolo Durand (Genoa, Italy); Giovanni Romeo (Genoa, Italy); Giorgio Bernardi (Paris, France)
The Worshop will focus on the new methodologies used to investigate human genetic diseases of unknown molecular basis. The strategies for identifying genes responsible for inherited disorders and cancer: the methods of analysis, cloning and sequencing of large chromosomal fragments, and the production of animal models of desired genotype will be addressed. The smallest human autosome, chromosome 21, will serve as a model system to illustrate the actual contributions that may derive from genome technology: the understanding of diseases of diverse genetic etiology, such as aneuploidies (down syndrome) mono/polygenic inherited conditions (alzheimer disease) and cancer.
SPEAKERS: W. Ansorge (Heidelberg, Germany); S. Antonarakis (Baltimore, USA); G. Bernardi (Paris, France); C Cantor (New York, USA); M. Capecchi (Salt Lake City, USA); A. Carrano (Livermore, USA); D. Cox (San Francisco, USA); A. deKlein (Rotterdam, The Netherlands); C. Epstein (San Francisco, USA); J. Gearhart (Baltimore, USA); R. Gemmill (Scottsdale, USA); Y. Groner (Rehovot, Israel); J. Gusella (Boston, USA); T. Hassold (Atlanta, USA); J. Ihle (Memphis, USA); E. Karson (Bethesda, USA); E. Lee (San Diego, USA); H. Lehrach (London, England); T. Papas (Frederick, USA); D. Patterson (Denver, USA); P. Pearson (Leiden, The Netherlands); A. Poustka (Heidelberg, Germany); G. Romeo (Genoa, Italy); D. Schlessinger (St. Louis, USA); N. Sacchi (Frederick, USA); N. Shimizu (Tokio, Japan); C. Smith (New York, USA); G. Stewart (Ann Arbor, USA); R. Tanzi (Boston, USA); D. Warburton (New York, USA); R. Worton (Toronto, Canada)
REGISTRATION AND INFORMATION: International School of Pediatric Sciences Telephone 039-10-383628; Direzione Scientifica 039-10-5636324; Istituto G. Gaslini 039-10-5636326; 16148 Genoa (Italy) Telefax 039-10-5636461
The Workshop is open to 40 participants. Preference will be given to applicants from Western Europe and Israel willing to present pertinent scientific contributions at the poster session. Deadline for application and poster abstract submission: February 28, 1989. **No Registration Fee.** Applicants must be able to cover their own travel and lodging expenses. (W5766)V

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Smith Kline & French Research Symposium

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12-14th April, 1989

CAMBRIDGE, U.K.
CO-CHAIRMEN: R. G. HILL AND T. J. RINK

This meeting will provide a general perspective on ion transport as well as a forum for the presentation of new developments. Each session focuses on a particular area of ion transport and will encompass mechanistic, molecular, physiological and pharmacological aspects of the topic.

Final Programme:

1. ION PUMPS

Chairman: I. M. Glynn, Cambridge
Speakers: G. Sachs, Los Angeles
S. J. D. Karlish, Rehovoth
C. W. Slayman, New Haven

2. IONS AND FLUID TRANSPORT

Chairman: A. W. Cuthbert, Cambridge
Speakers: J. C. Ellory, Oxford
R. Greger, Frieburg
Q. Al-Awqati, New York

3. VOLTAGE-GATED CHANNELS

Chairman: A. F. Huxley, Cambridge
Speakers: B. Hille, Seattle
T. B. Bolton, London
P. Hess, Boston
R. W. Aldrich, Stanford

4. RECEPTOR-OPERATED CHANNELS

Chairman: L. L. Iversen, Harlow
Speakers: T. J. Rink, Welwyn
M. L. Mayer, Bethesda
E. Barnard, Cambridge

5. MODULATION OF ION CHANNELS

Chairman: D. Noble, Oxford
Speakers: H. Reuter, Berne
A. Dolphin, London
F. Hoffman, Saarlandes

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6. EVENING WORKSHOP

on Models of ion permeation through channels. Discussion led by D. T. Edmonds, Oxford and S. B. Hladky, Cambridge

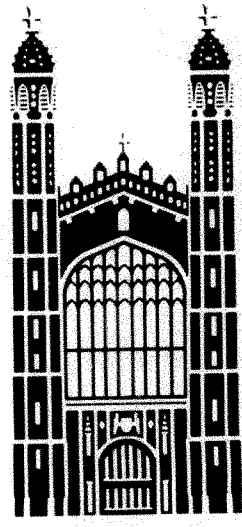
Poster session: Posters are invited from participants and abstracts will be included in the Symposium Proceedings. Closing date for receipt of abstracts is *13th March 1989.*

Registration: Details are available from Ms M. H. Hancock, Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts AL6 9AR. Telephone: (0707) 325111, Ext. 3894. The registration fee of £180.00 (£90.00 for registered undergraduate and graduate students) includes welcome buffet, conference dinner, all other meals, accommodation for three nights and a copy of the Symposium Proceedings.

Sessions are in the Corn Exchange; accommodation in King's College.

Organising committee: C. D. Benham, D. J. Keeling, B. K. Leigh. Smith Kline & French Research Ltd., The Frythe, Welwyn, Hertfordshire AL6 9AR. Telephone: (0707) 325111.

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product review

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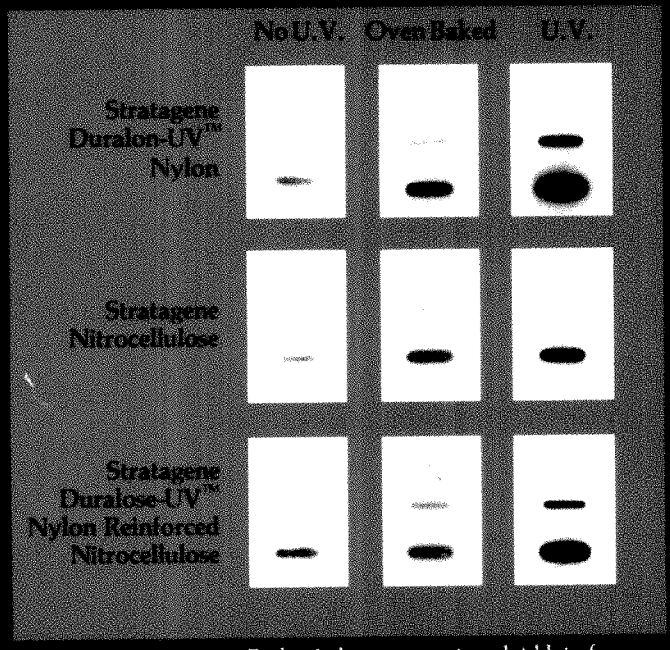
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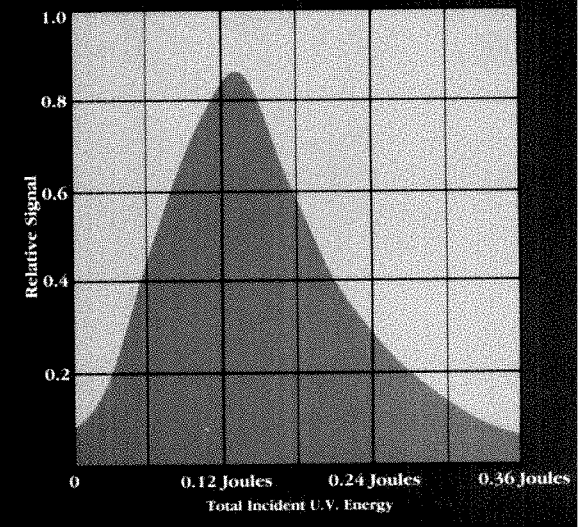
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Each window represents a slot blot of lambda DNA applied at concentrations equivalent to 0.1, 1.0, and 10 copies per mammalian genome diluted in 5 micrograms of calf thymus DNA and hybridized with a 32P-labeled lambda DNA probe. The film was developed after an overnight exposure.



The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The peak signal intensity is at approximately 0.12 Joules. The significant drop in signal intensity at energies below and above 0.12 Joules demonstrates the limited optimal range for U.V. treatment.

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Some of the international meetings scheduled for 1989 are:

Unexplained Infertility: Basic and Clinical Aspects
Rome, Italy/March 7-8
Scientific Organization: G. Benagiano (I), K.J. Catt (USA) and G. Spera (I)

Symposium on the Pathogenesis and Control of Viral Infections
Beijing, Republic of China/April 24-26
Scientific Organization: F. Aiuti (I), Z. Zonghan (PRC) and S. Guoxian (PRC)

th Workshop on Development and unction of the Reproductive Organs
ouraine, France/May 23-25
cientific Organization: N. Josso (F)

1st International Congress on G.I.F.T.: from Basics to Clinics
Rapallo, Italy/June 8-10
Scientific Organization: R.H. Asch (USA) and L. De Cecco (I)

Membrane Technology in Clinical Pathology, Biochemistry and Pharmacology
L'Aquila, Italy/June 19-23
Scientific Organization: R. Verna (I), R.P. Blumenthal (USA), J.A. Hannover (USA) and R.P. Garay (F)

Cardiovascular and Neurological Function and Ovarian Secretions
Dubrovnik, Jugoslavia
Aug. 31-Sept. 1
Scientific Organization: F. Naftolin (USA)

Establishment of a Successful Human Pregnancy
Cambridge, U.K./September 21-23
Scientific Organization: R.G. Edwards (UK)

Developmental Endocrinology
Geneva, Switzerland/October 23-24
Scientific Organization: P.C. Sizonenko (CH) and M. Aubert (CH)

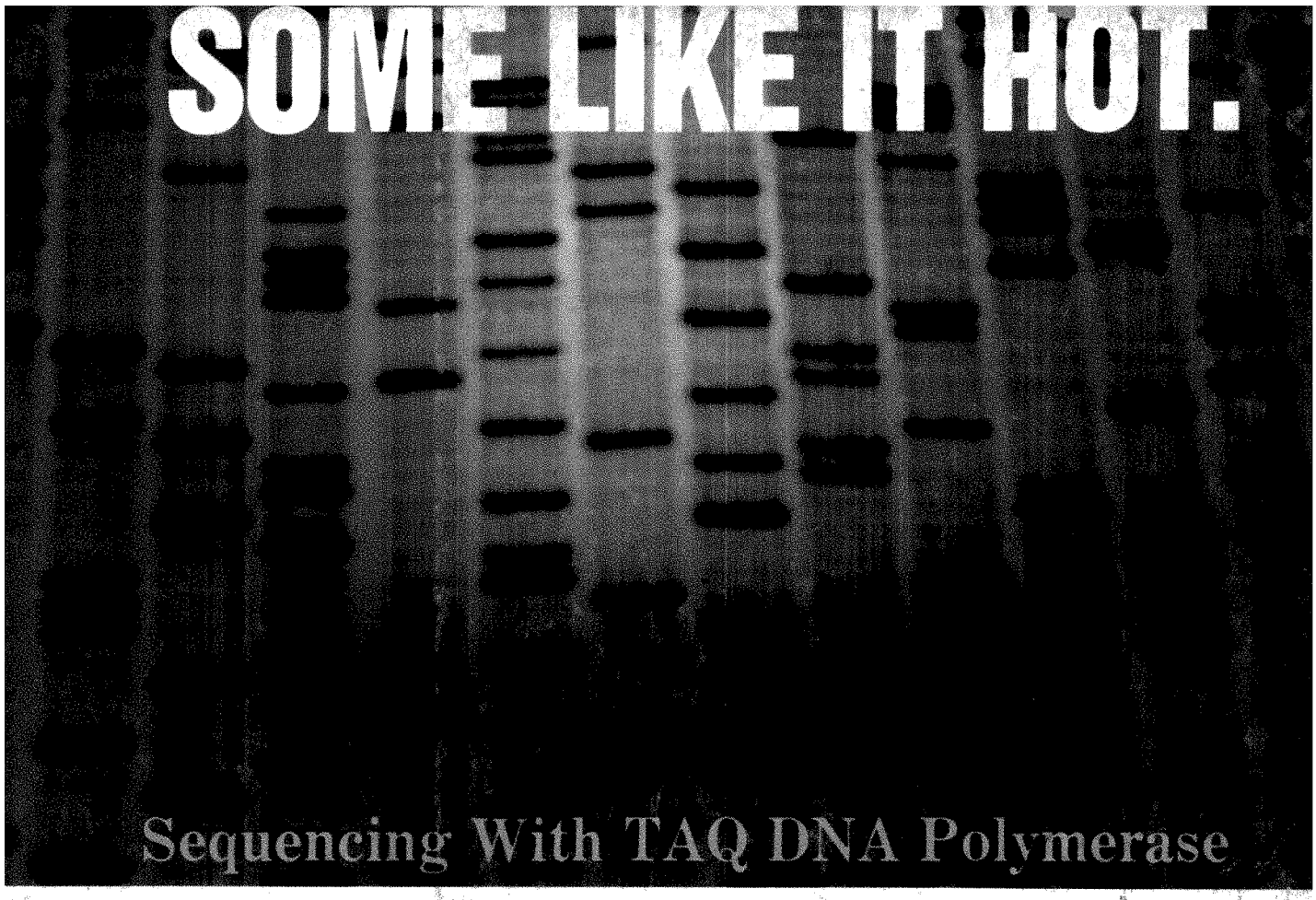
Advanced Course in Molecular Pathology and Biotechnology
Rome, Italy/November 6-10
Scientific Organization: L. Frati (I)

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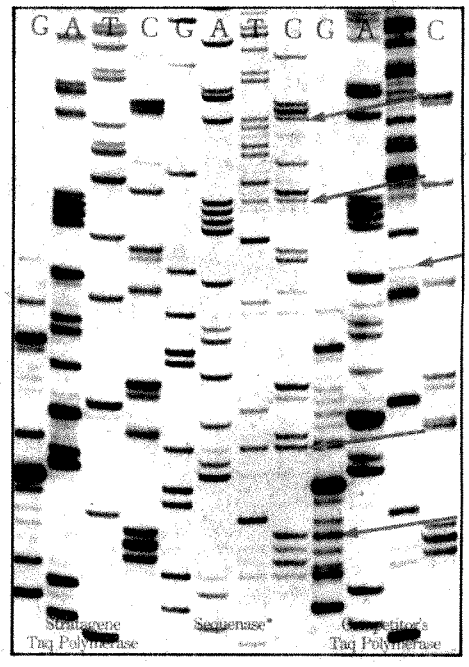
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Dr. Maryann Zounes of Syntro Corporation has independently compared Stratagene's Taq Polymerase with Sequenase* (modified T7 DNA polymerase) and a competitor's Taq polymerase. Double stranded supercoiled DNA was prepared using the boiling miniprep procedure. Sequenase* reactions were performed according to the manufacturer's instructions.

Arrows highlight Sequencing artifacts.

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The accompanying figure illustrates the ability of Stratagene's Taq polymerase to sequence a double stranded DNA region in which Sequenase* and a competitor's Taq polymerase generate multiple artifact (arrows). Stratagene's Taq polymer proceeds through these regions with little difficulty. Overall, Taq polymerase provides substantially more information in artifact-prone regions than either the Klenow fragment or Sequenase*.

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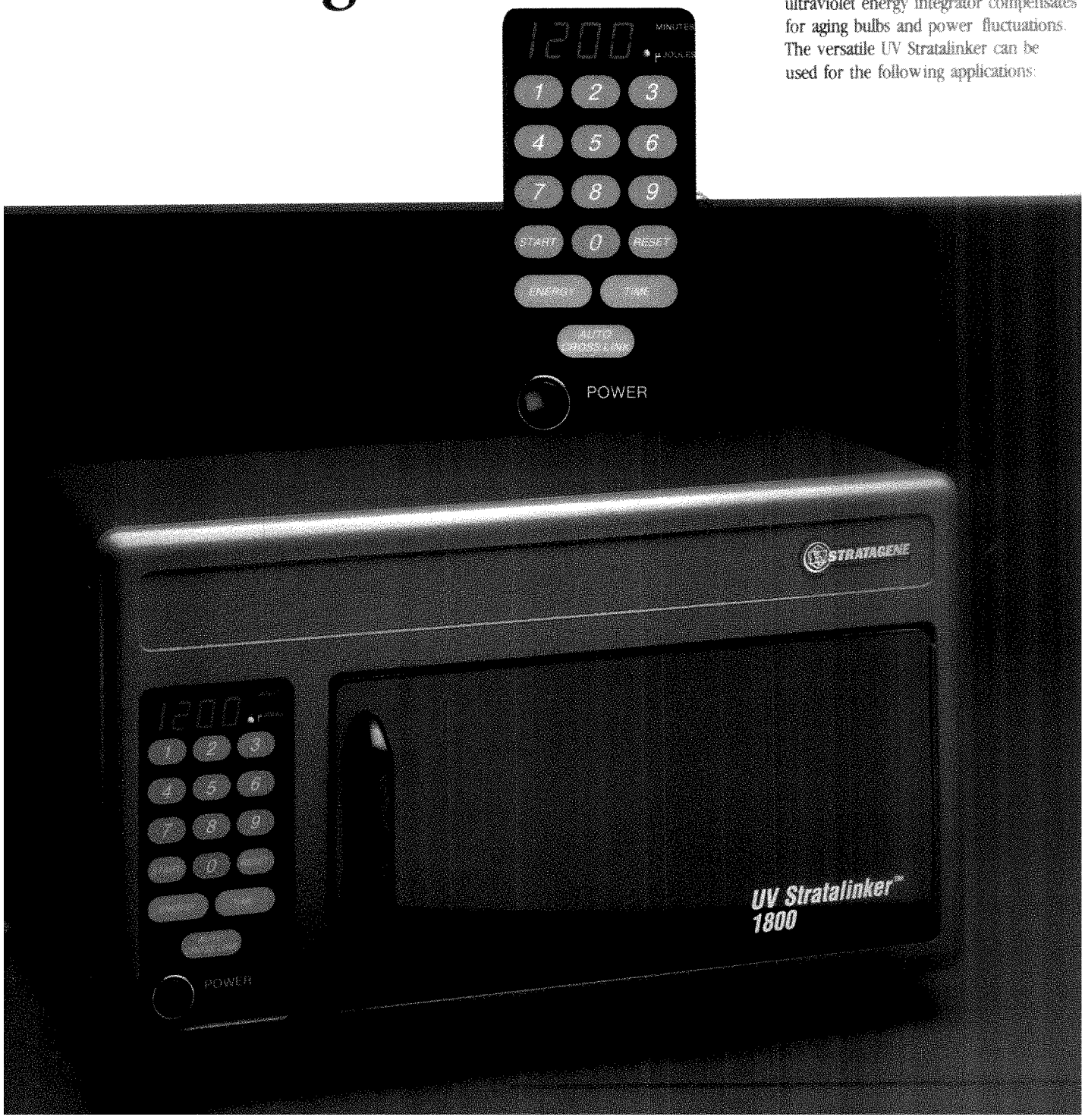
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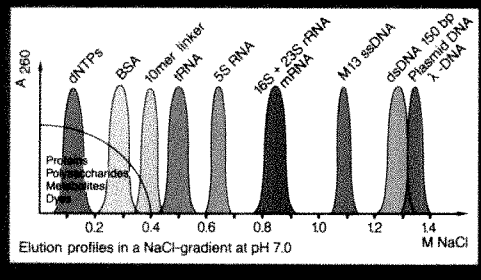
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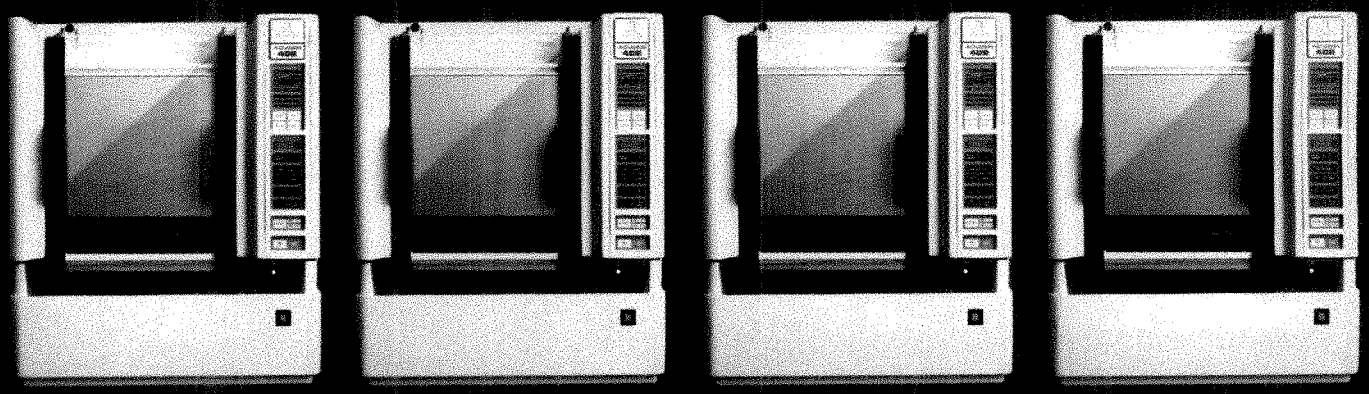


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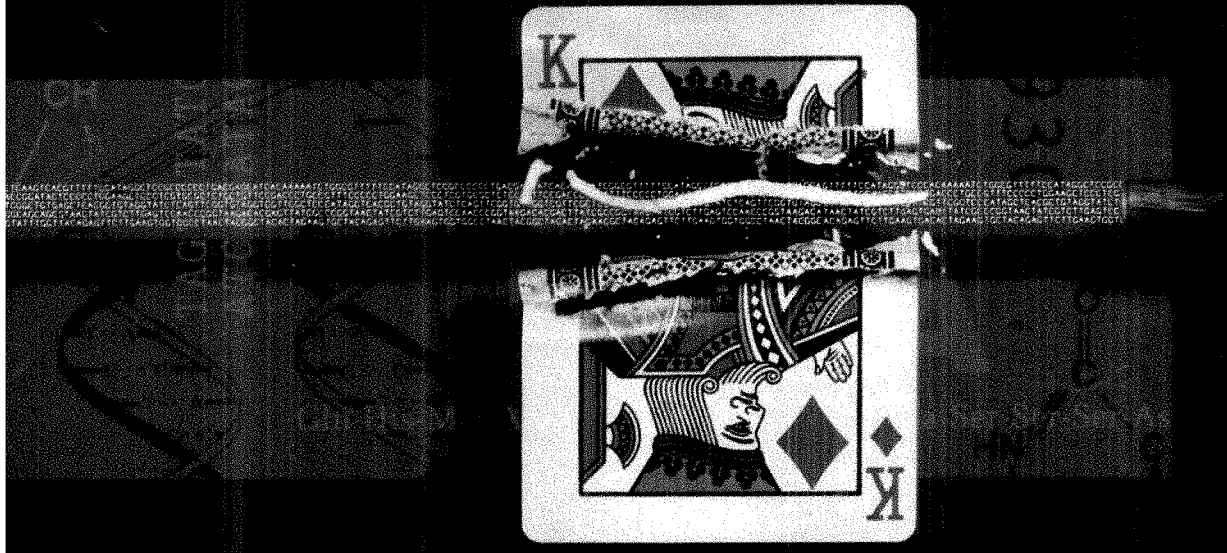
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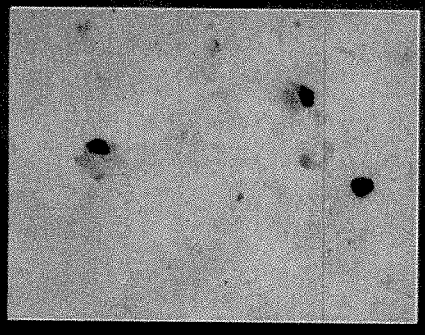
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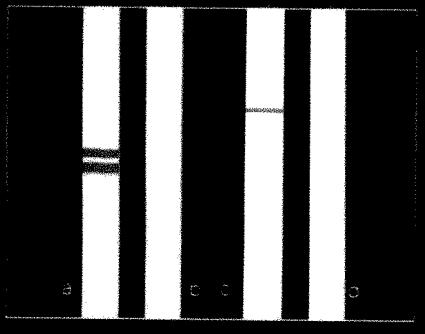
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← *In situ* hybridization of cytomegalovirus (CMV)-infected adrenal gland using biotinylated entire CMV genome, rabbit anti-biotin, AuroProbe One and IntenSE. (Volkers, van den Brink & Houthoff, Amsterdam)

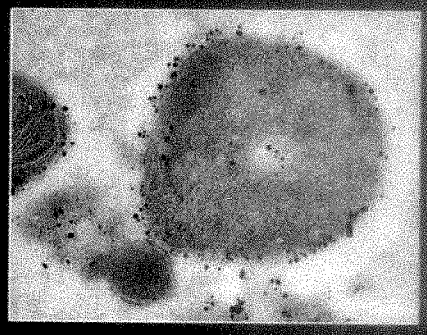


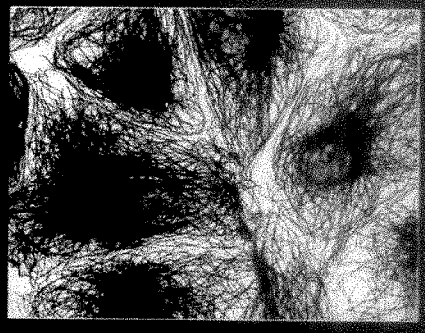
← Blot of total extract of Hela cells and blot of partially purified nuclei of rat liver reacted respectively with rabbit anti-tubulin (a) and rabbit anti-actin (c) followed by AuroProbe One and IntenSE. Negative controls (b, d).

Cryosectioned synaptosomes reacted with rabbit anti-B50, AuroProbe One and IntenSE. (van Lookeren-Campagne & van Bergen en Henegouwen, Utrecht) →

PtK$_2$ cells reacted with rabbit anti-tubulin, AuroProbe One and IntenSE →

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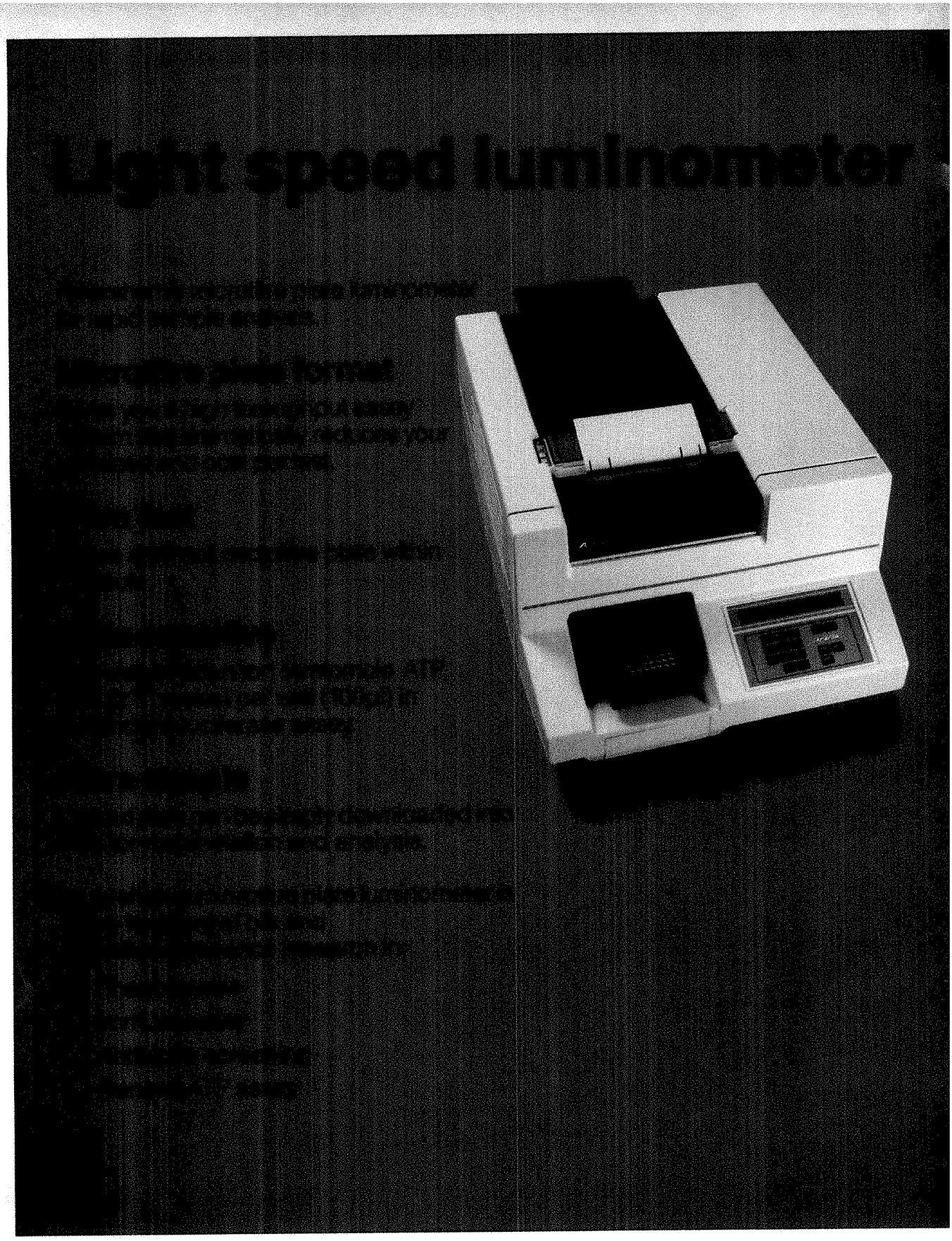
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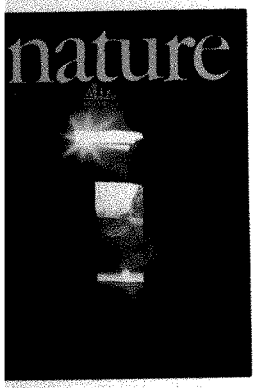


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nature

26 January 1989
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The winter solstice Sun shining through the 'roof-box' of the Neolithic passage graves at Newgrange, Ireland, illuminating the main chamber. When the monument was constructed, 5,000 years ago, it may have been used to trace the path of the Sun during the solstice sunrise. See page 343. Cover photo: Claire O'Kelly.

THIS WEEK

Law inaction

Recent judgements in the British courts raise doubts about whether established patents law can cope with new products made by recombinant DNA technology. The antithrombosis agent tissue plasminogen activator is the case in point. Page 317.

Electrons in charge

The discovery of a new family of superconducting copper oxides in which the carriers of the supercurrent are electrons — rather than electron vacancies or 'holes' — has important implications for theories of high-temperature superconductivity, pages 345 and 306.

New antibiotics?

A synthetic peptide, modelled on the reactive centre of a human cysteine proteinase inhibitor, effectively blocks the growth of group A streptococcal strains, page 385.

Fossil nebula

The temperature of the massive central star in nebula N44C in the Large Magellanic Cloud is far too low to account for the high excitation emission from the surrounding gas. Is the nebula an 'extinct' luminous X-ray binary? See page 337.

Spadetail mutant

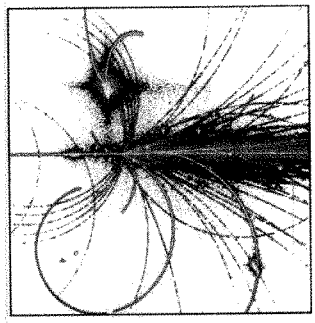
A newly discovered mutant zebrafish should be invaluable in studies of embryo development. An abnormality of mesodermal cell migration changes the position and with it the fate of the cells that normally form trunk somites, page 358.

Before green plants

Are prochlorophytes related to a prokaryotic ancestor of chloroplasts? Sequences of the gene for a component of photosystem II support this phylogeny, but those of 16S rRNAs do not. Pages 380, 382 and 304.

Nuclear matters

The behaviour of dense nuclear matter, whether in heavy atomic nuclei, inside neutron stars or during the early moments of the



Universe, may soon be better understood. Review Article, page 319.

Hard times

Cubic boron nitride — second only to diamond in hardness — has been studied at high pressure using three different methods that show remarkable agreement, page 349.

DNA from RNA

The essential RNA component of the telomerase that synthesizes the telomeric repeats of *Tetrahymena* chromosomes contains the complement of the repeats and thus acts as an RNA template for DNA synthesis, pages 331 and 303.

Guide to Authors

See page 386.

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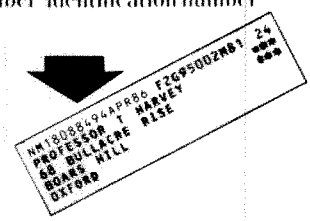
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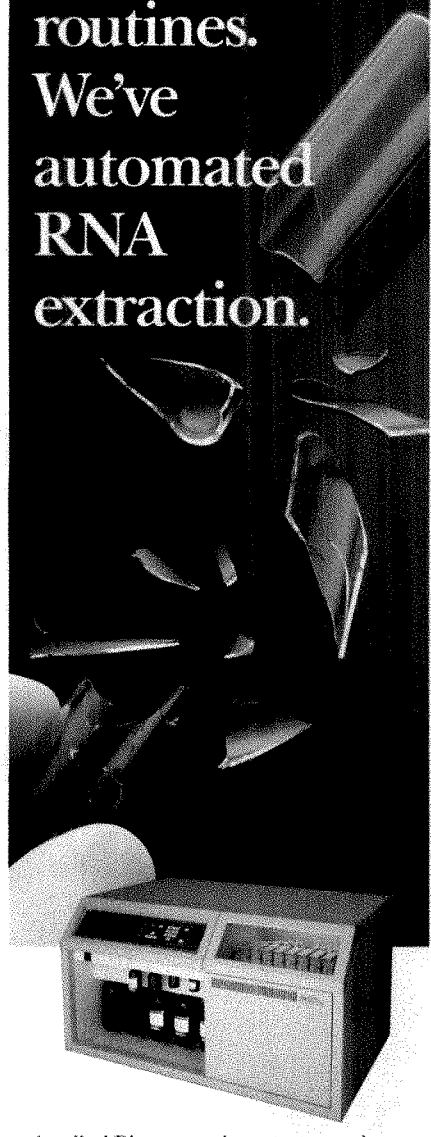
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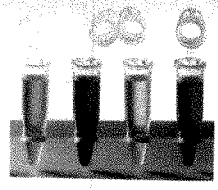
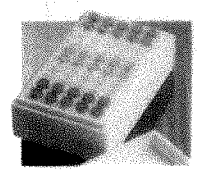
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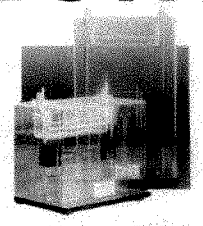
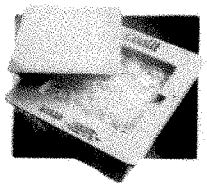
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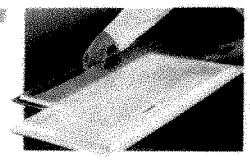
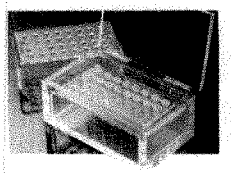
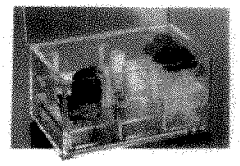
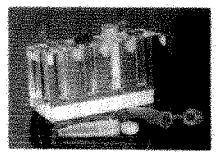
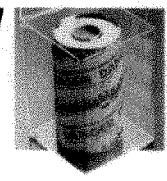
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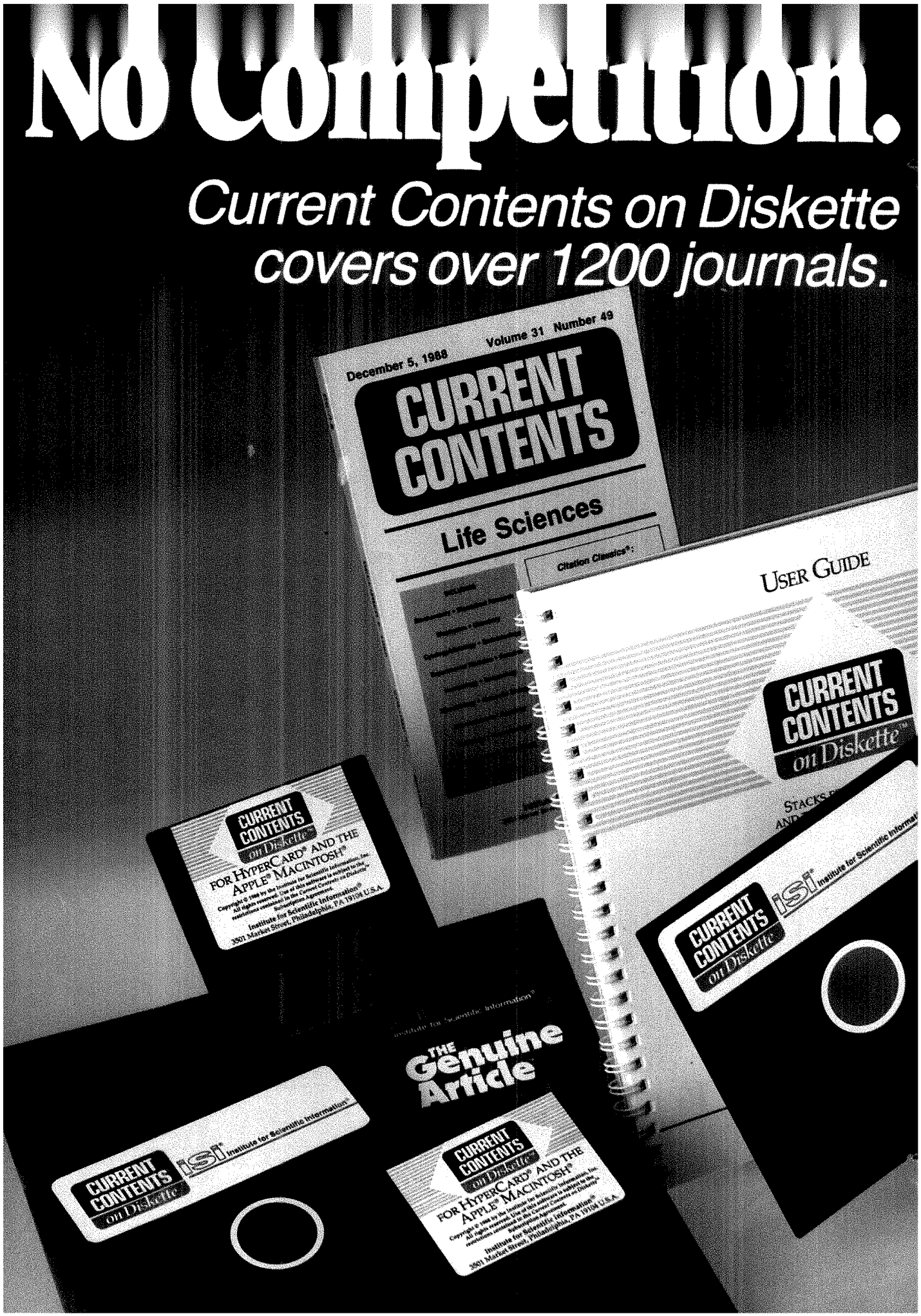
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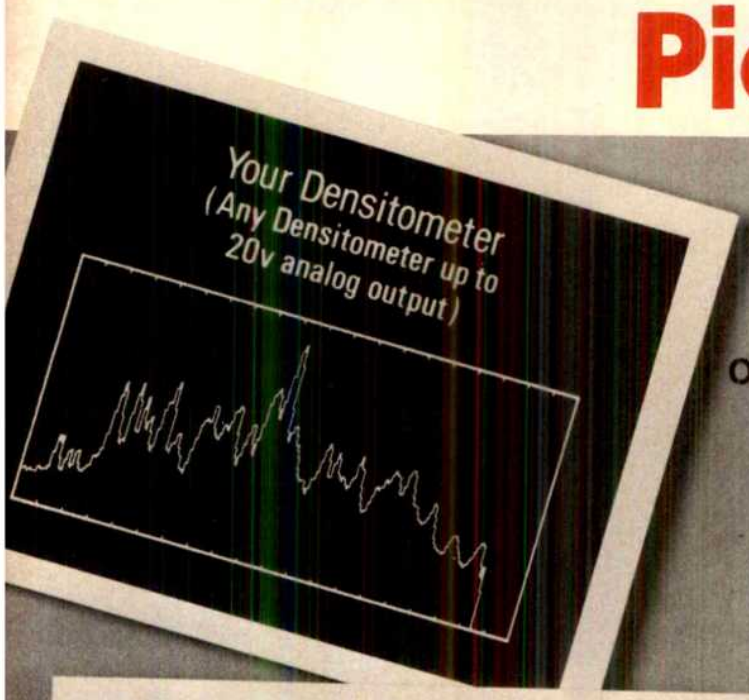
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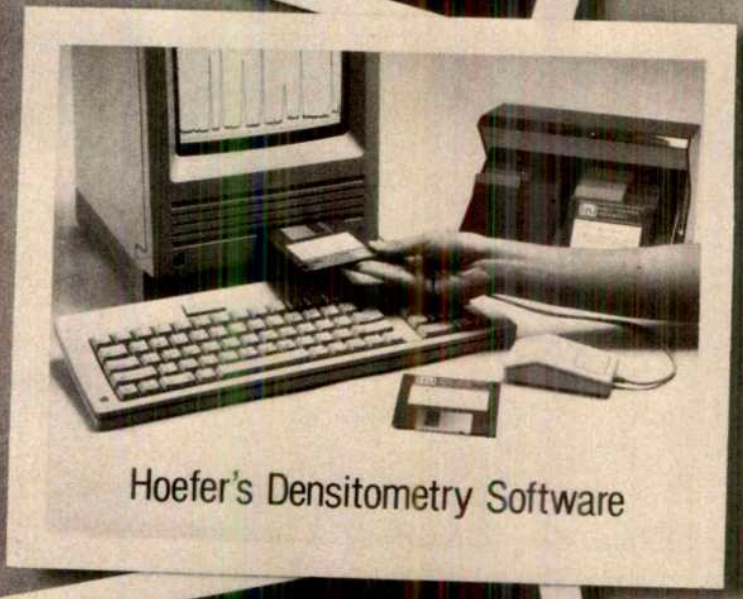
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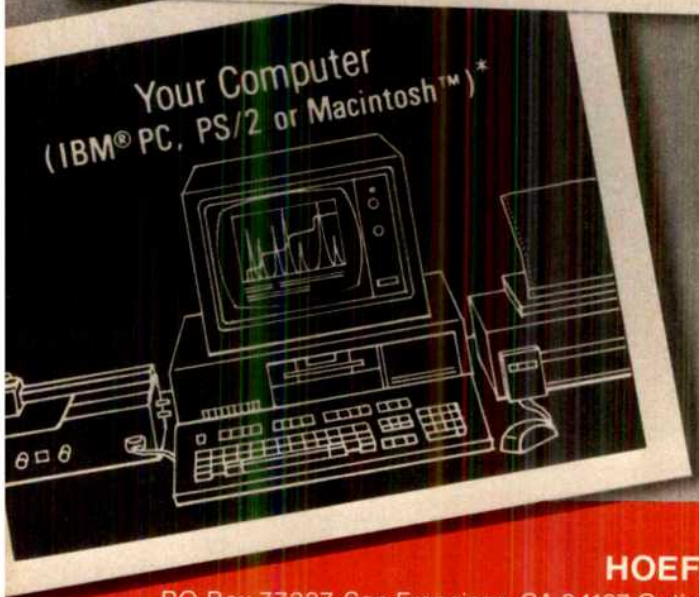
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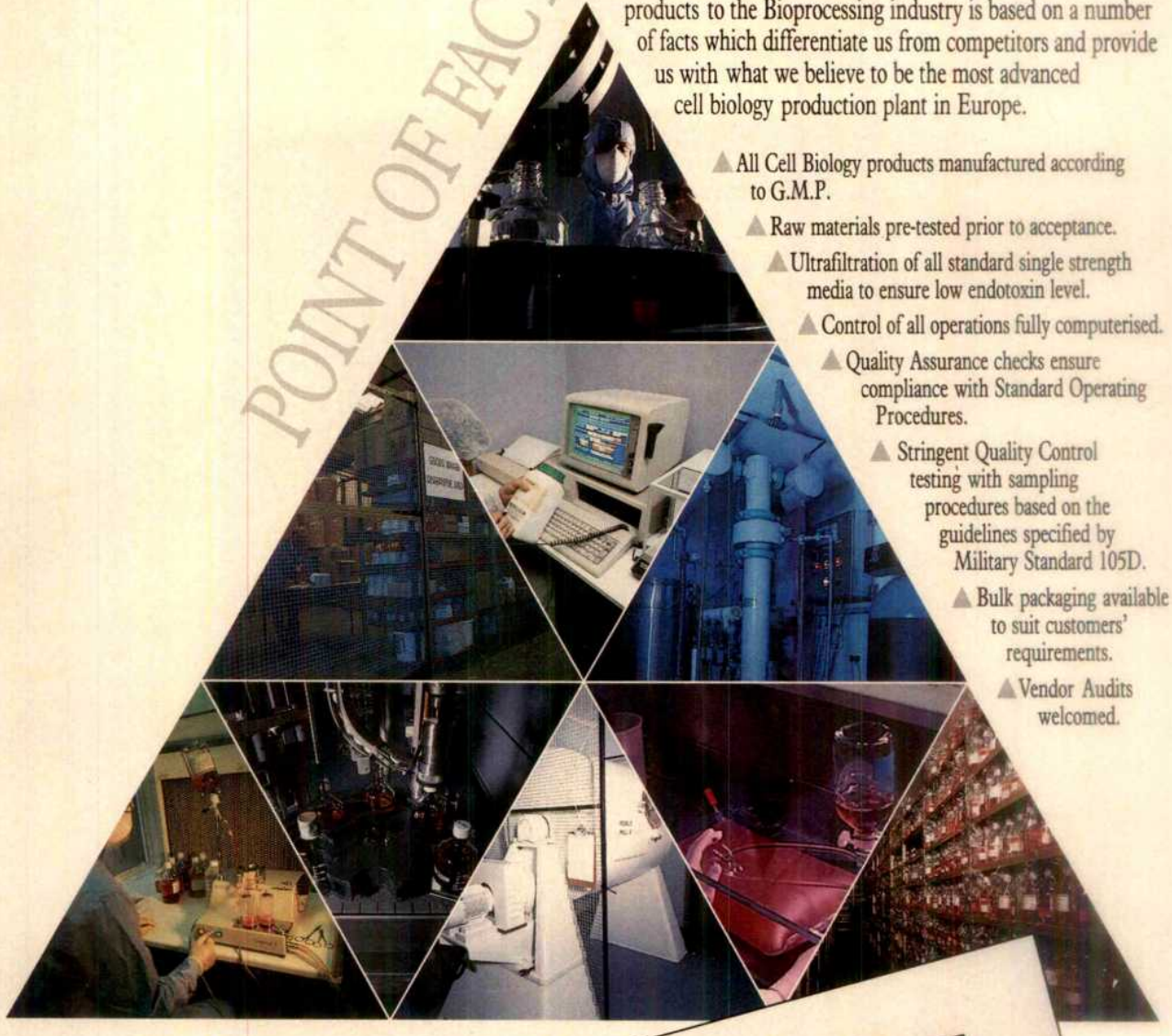
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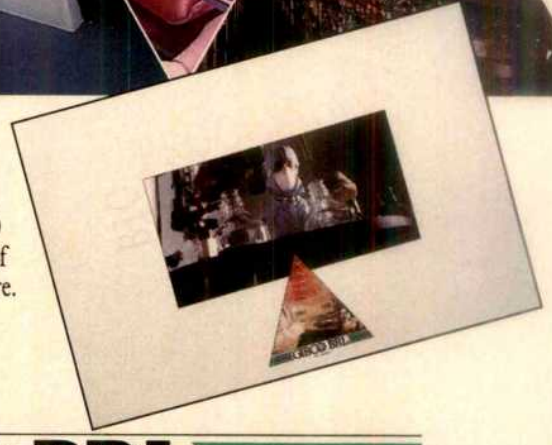
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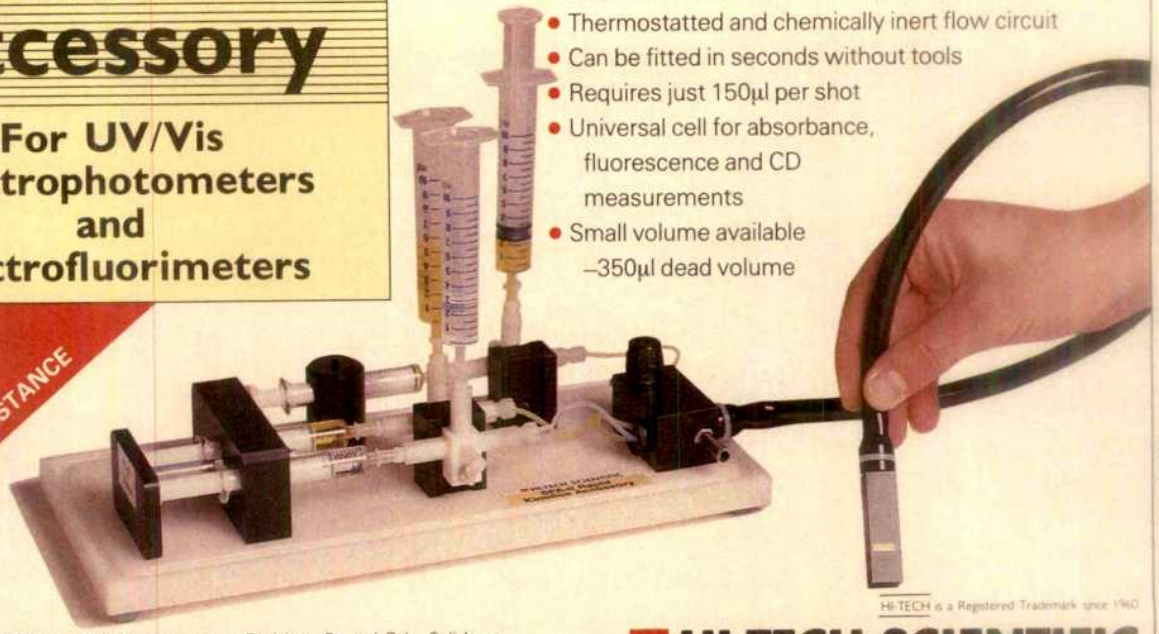
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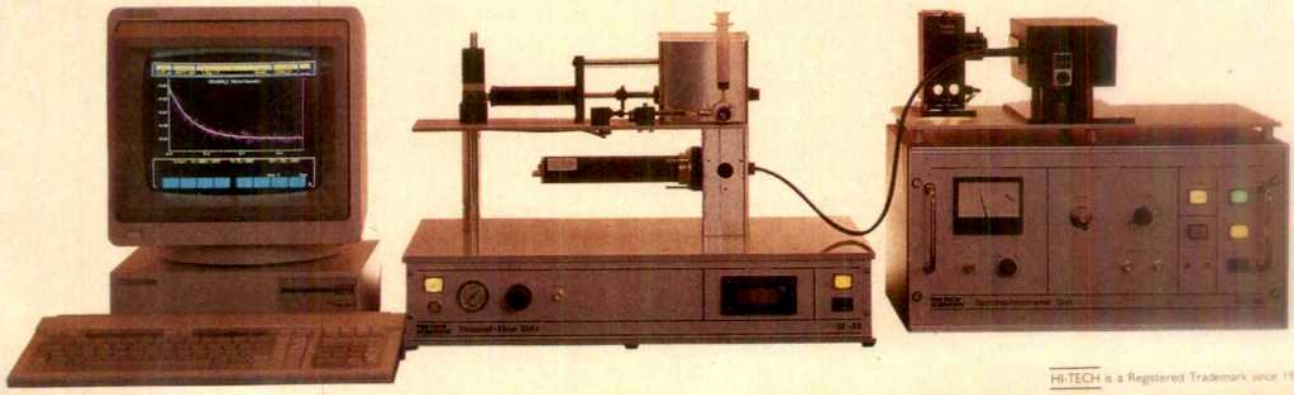
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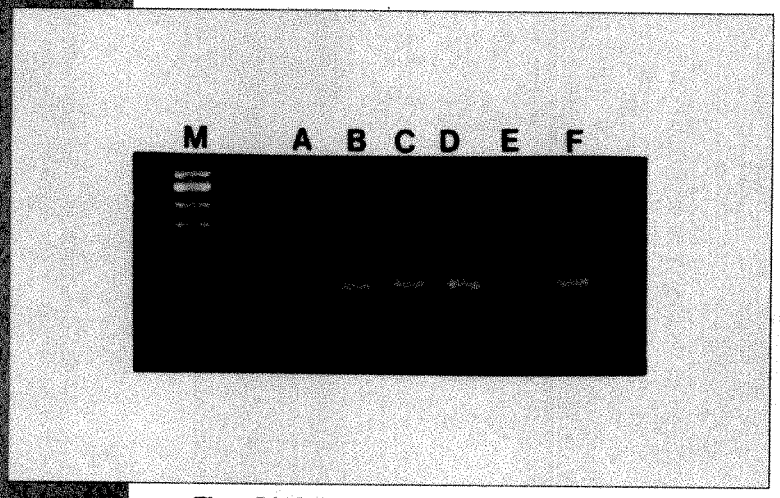
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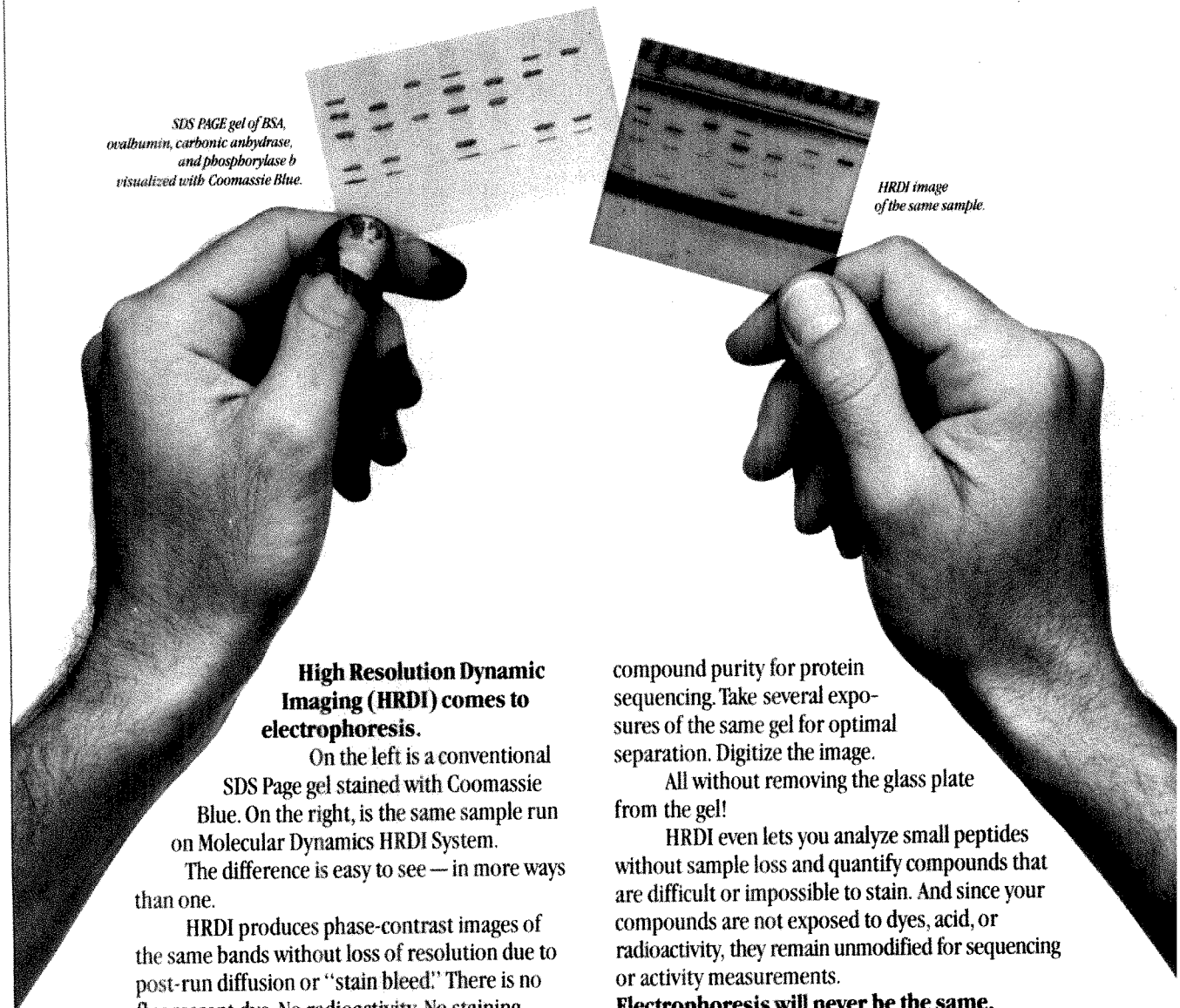


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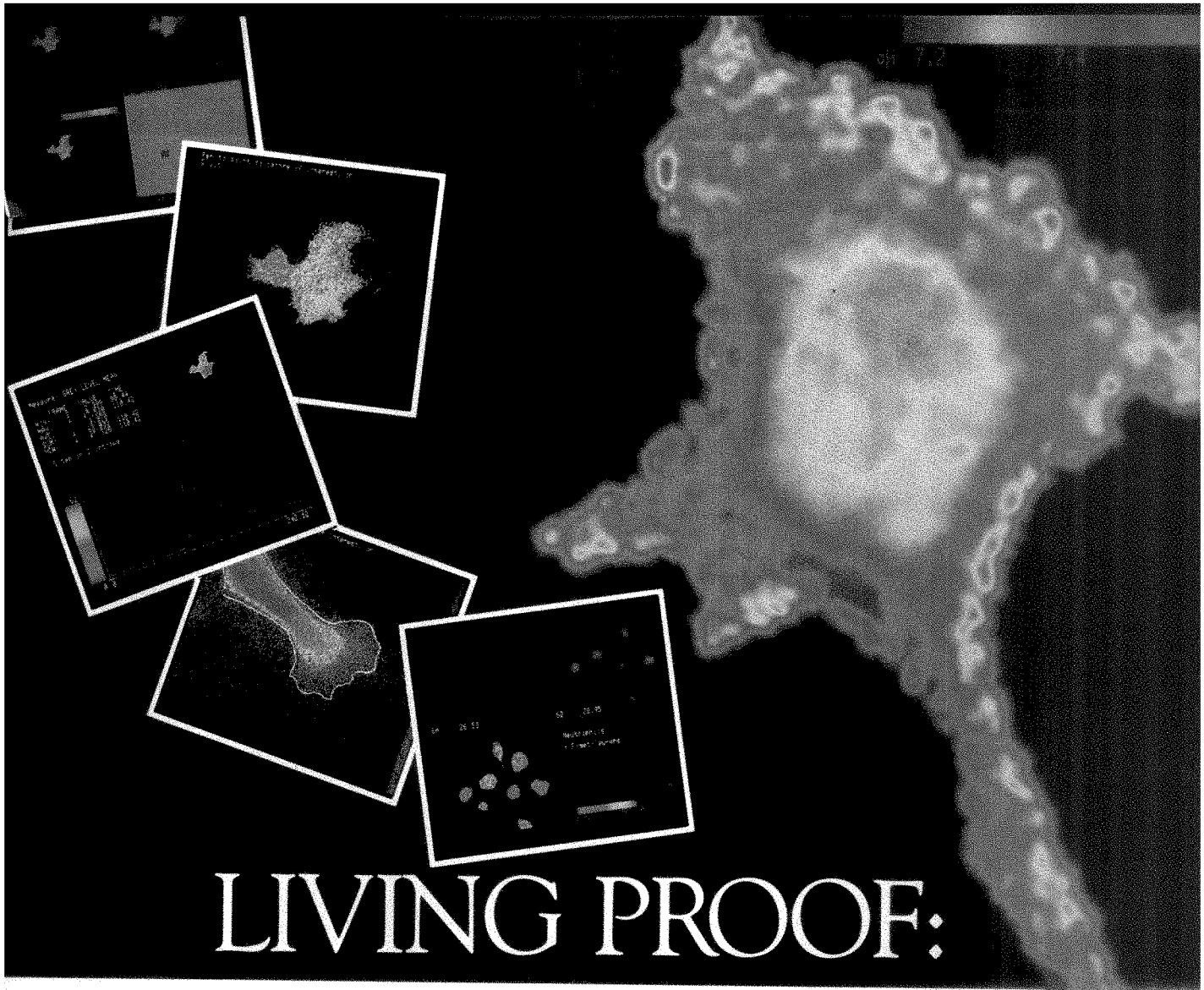
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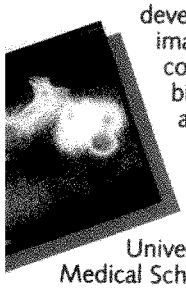


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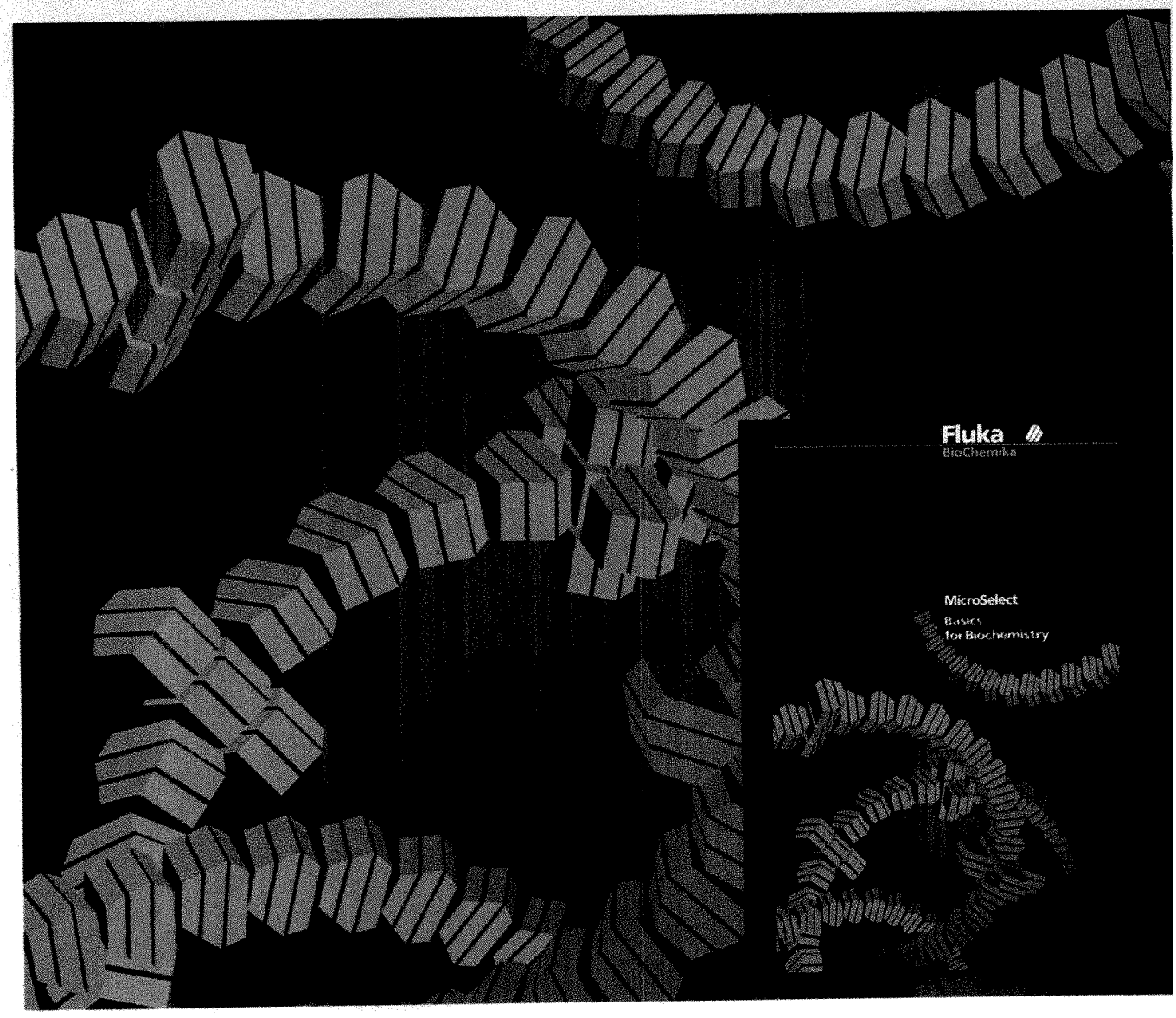
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Significant advances are being made in the cytogenetic and molecular genetic characterization of benign and malignant neoplastic cells. Tumor-specific chromosomal changes identify the locations of genes critical to malignant transformation. Molecular analyses of these genes or DNA sequences provide a more detailed understanding of their structure and function in both normal and neoplastic cells. Tumor-associated chromosomal and DNA alterations serve as increasingly important diagnostic and prognostic markers. Efforts to identify genes that determine inherited susceptibility to cancer are progressively more successful.

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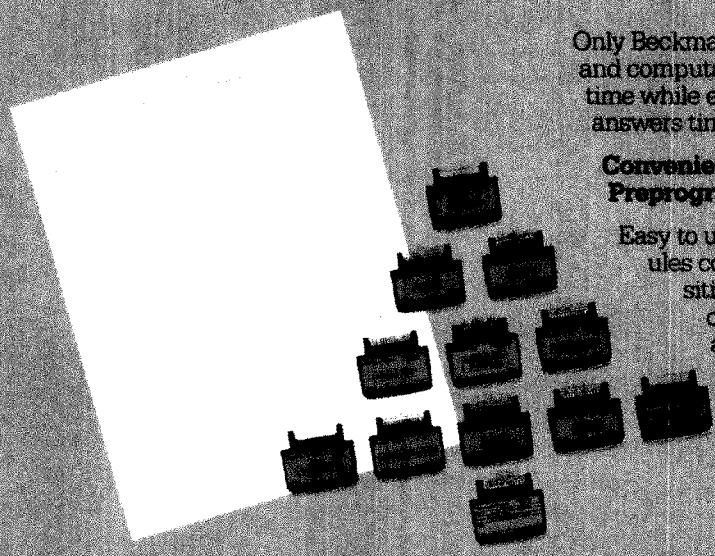
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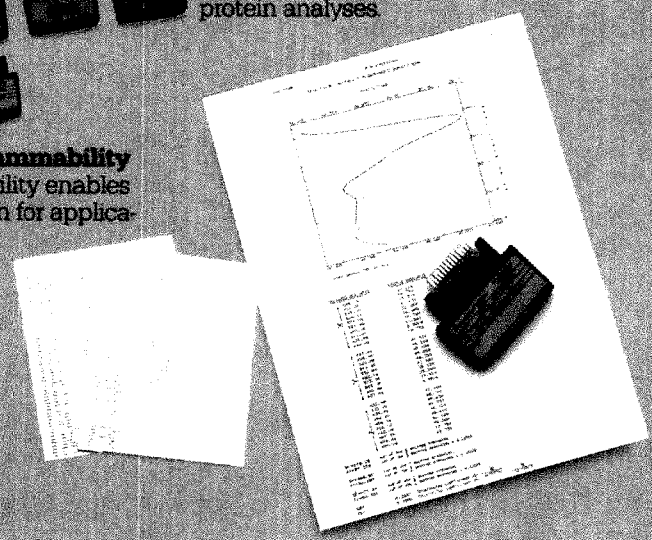
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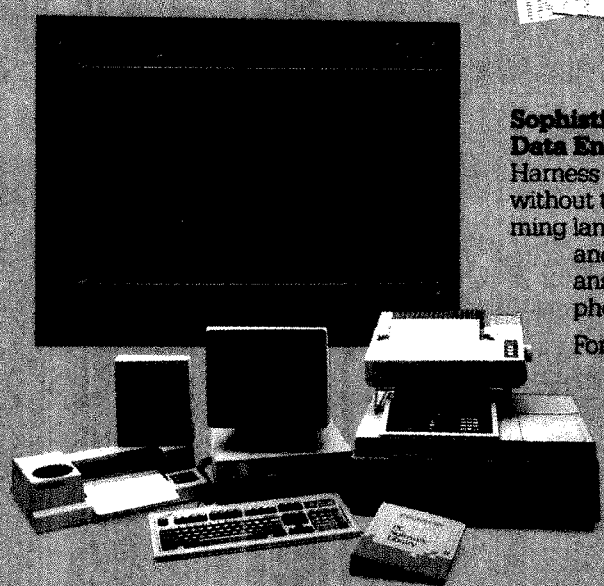
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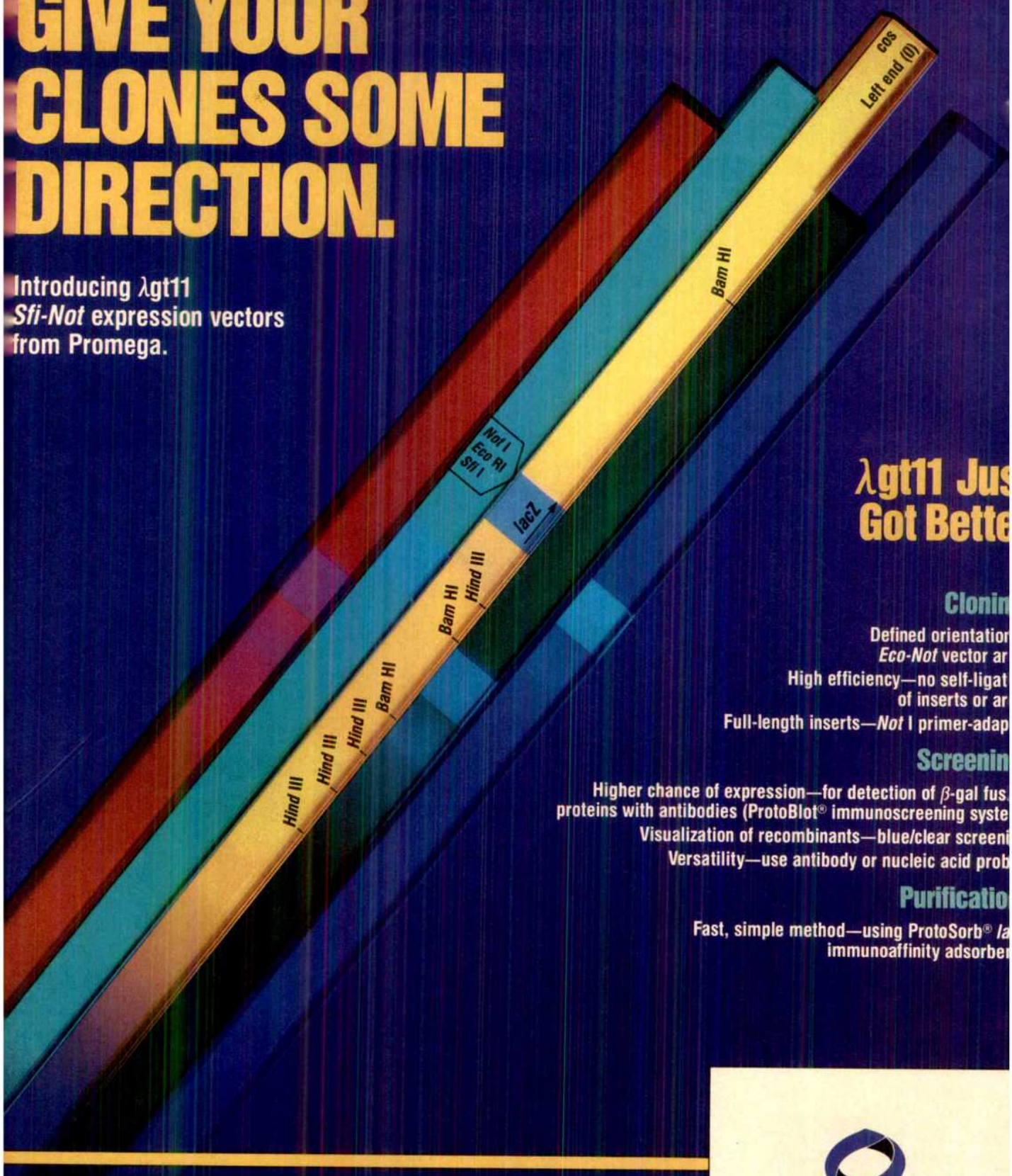
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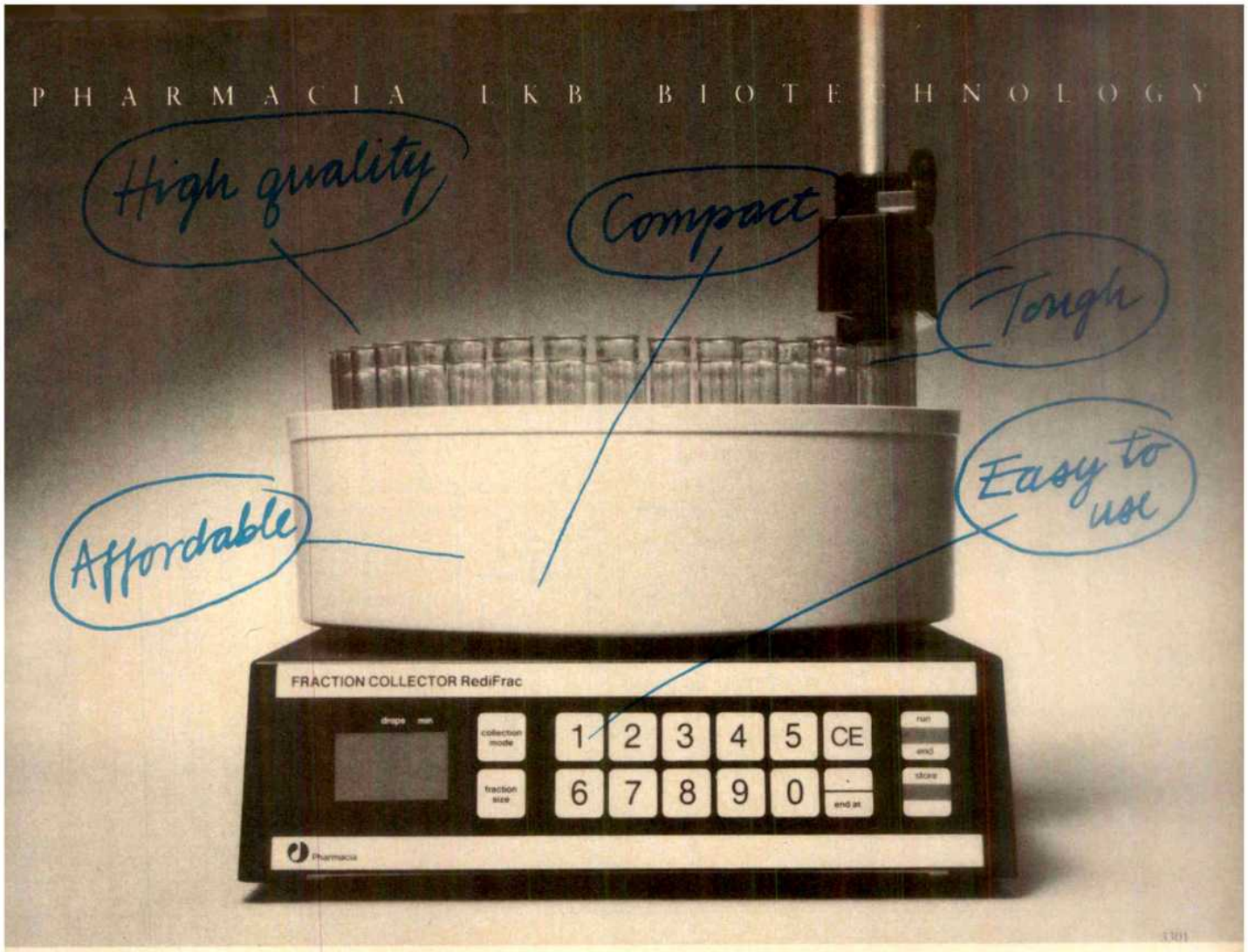
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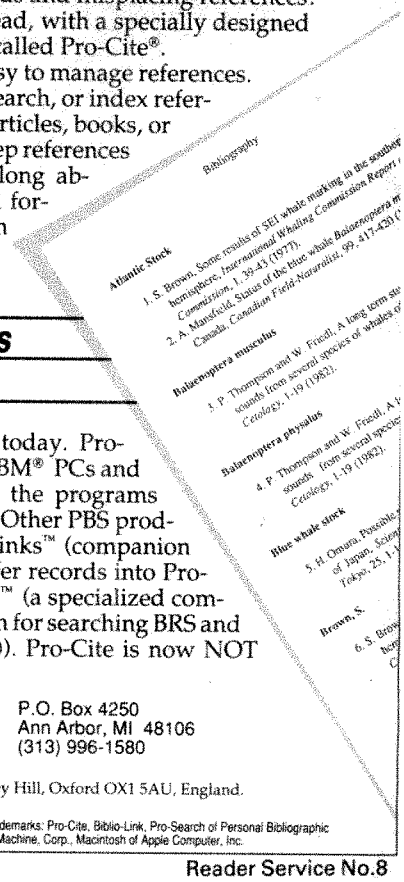
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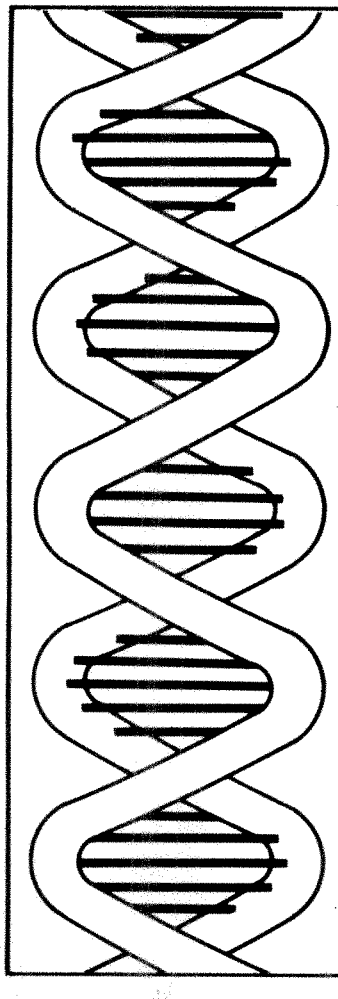
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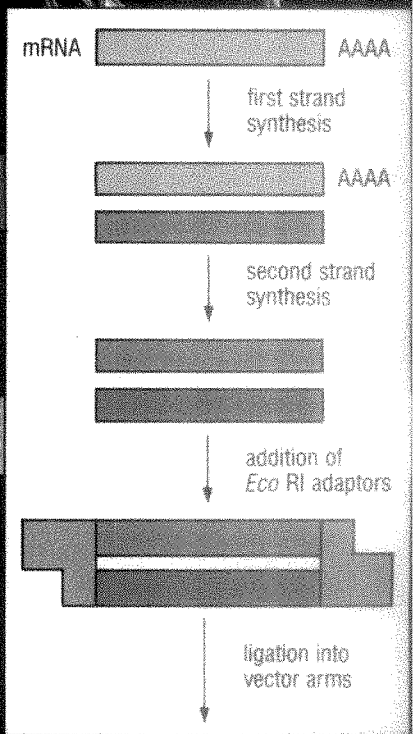
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S.J. Benkovic : The Pennsylvania State Univ. (U.S.A.)
T. Beppu : The Univ. of Tokyo (Japan)
F.T. Hong : Wayne State Univ. (U.S.A.)
K. Imai : Osaka Univ. (Japan)
S. Nishimura : National Cancer Center (Japan)
H. Okada : Osaka Univ. (Japan)
A.R. Rees : Univ. of Oxford (U.K.)
G. Winter : MRC (U.K.)

Workshop I:
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P. Argos : EMBL (F.R.G.)
N. Go : Kyoto Univ. (Japan)
D.G. George : NBRF (U.S.A.)
M. Kanehisa : Kyoto Univ. (Japan)
J.L. Markley : Univ. of Wisconsin (U.S.A.)
T. Ooi : Kyoto Women's Univ. (Japan)
A. Tsugita : Science Univ. of Tokyo (Japan)

Workshop II:
SECRETION AND TRANSLOCATION OF PROTEIN

K. Ito : Kyoto Univ. (Japan)
M. Kikuchi : Protein Engineering Research Institute (Japan)
S. Mizushima : The Univ. of Tokyo (Japan)
W. Neupert : Univ. München (F.R.G.)
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The Lynen Lecture
I. Neher and B. Sakmann

Monday PM
Molecular Biology of Ion Channels
W.A. Catterall, D.M. Fambrough, Y.N. Jan

Monday PM
Receptor-Based Diagnostics
S.J. Enna, C.D. Johnson, J. Ramachandran

Tuesday AM
The Distinguished Service Award
W. Gilbert

Tuesday AM
Regulation of Gene Expression
M.G. Rosenfeld, R.H. Scheller

Tuesday AM
Design of Receptor Ligand Pharmacophors
M.B. Bolger, A.D. Strosberg, H.I. Yamamura

Tuesday PM
Cell Recognition and Development
J. Bixby, U.J. McMahan, M. Schachner

Tuesday PM
Nerve Growth Regeneration and Transplantation
A.J. Aguayo, F.H. Gage, F.F. Hefti, J.R. Sladek

Wednesday AM
Molecular Basis of Memory and Learning
D. Alkon, W. Gispen, P. Andersen

Thursday AM
Special Achievement Award
Osamu Hayaishi

Sorting and Localization of Proteins
G.A. Banker, R.B. Kelly, R.L. Rotundo

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Neuropeptides
F.E. Bloom, L.L. Iversen, S.J. Watson

Thursday PM
Second Messengers
K.J. Catt, R. Llinas, H. Rasmussen

Thursday PM
Sensory Systems and Transduction
A.J. Hudspeth, S.H. Snyder, C. Zuker

Friday AM
Molecular Aspects of Neurological Diseases
H.F. Epstein, J.B. Martin, D.L. Price, D.S. Gerhard

THE SECOND ANNUAL NEURAL SYSTEMS SYMPOSIUM: COMPUTER NEURAL NETWORKS: A CRITICAL OVERVIEW

Tuesday
Pattern Recognition as Motivating Neural System Research
David Bendel Hertz

Neural Self Organization
Stephen Grossberg

A Neural Network Architecture For Preattentive Vision
Ennio Mingolla

Adaptive Hand-Eye Coordination
Michael Kuperstein

Optical Realization of Pattern Recognition
Dana Anderson

Stochastic Neural Net and Optical Learning Machines
Nabit Farhat

Neural Networks for Speech Recognition
Richard Lippmann

Wednesday
Instantiation Schemes for Pattern Recognition
Yi-Tzu C. Chien

Optical Realizations of Bidirectional Associative Memories
B. Keith Jenkins

Mapping Multi-Layered Neural Networks for Pattern Recognition Onto Multi-Processors
Genevieve Cerf

Available Recognition Schema
Leon Cooper

Future Directions For Neural Networks
Luis Glaser

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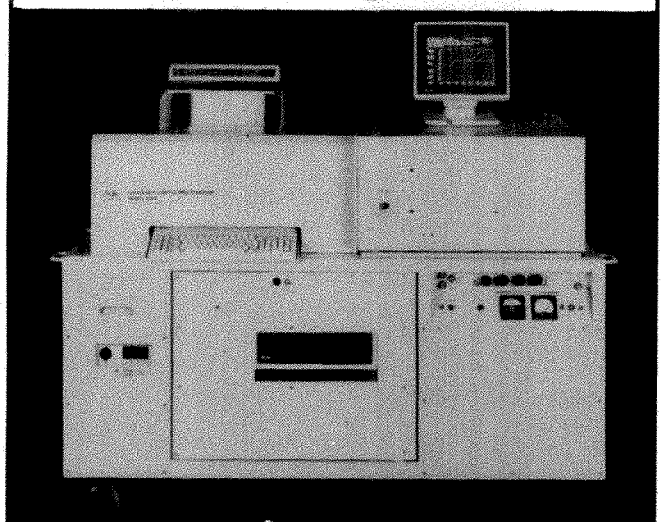
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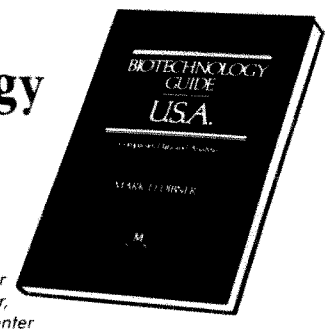
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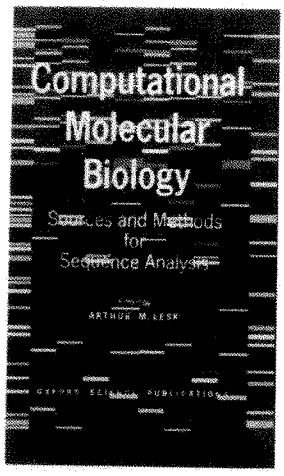
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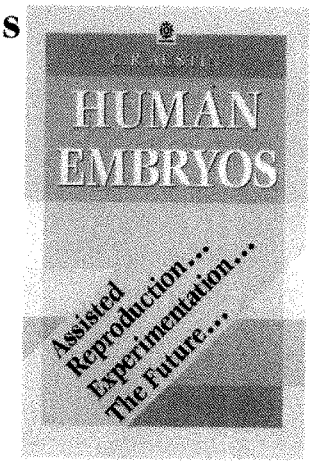
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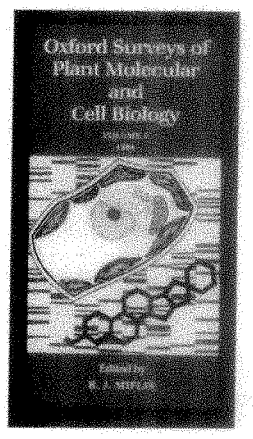
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nature

Safeguarding British research

Recent improvements of the climate in British research do not imply that the dangers have passed. Indeed, the most serious — complacency — is more serious now than ever.

SURPRISING as it may seem, the morale of the British research enterprise may be on the mend, if only slowly. There are several explanations, not the least of which is that the spate of bad news has abated to a mere trickle. Laboratories are still being closed — a meat research station a few weeks ago, now a couple of veterinary schools (see page 294) — but to those still with posts, the prospect that most institutions would be shrunken images of their present, let alone their past, selves seems to have receded. And there is to be some extra cash. In the financial year that begins in April, the government's direct subvention of basic research will increase by £95 million to a total of £850 million. Indeed, potential recipients of the extra funds might by now have known whether anything extra is likely to come their way if the government's announcement of how the total budget will be allocated between the research councils and other spending agencies, originally planned for earlier this week, had not unaccountably been postponed.

In the circumstances, there is a danger that alarm, itself begotten of complacency, will breed a further round of indifference. At a time when even British high-energy physics has been told (in last week's corporate plan of the Science and Engineering Research Council — see page 296) that there is no "wish to reduce the provisions for nuclear physics further", it may be all too tempting to suppose that the passage of time will mend the damage done to British research in the past decade, in due course even providing a platform for resurgence. But in reality, passivity of that kind will invite only further trouble. If there is now to be a breathing space, the research community should use it as an opportunity for healing wounds and for ensuring that the privations of the past decade will not recur.

It is important, first, to understand what has happened. The mere fact that laboratories have been closed and their members dispersed or put out to grass is in one sense water under the bridge. The process of contraction may have seemed arbitrary at the time, and unjust to many of the people who lost their jobs, but nobody would now recreate the same laboratories even if practical problems since brought to light cannot now as easily be solved. (The agriculture and food ministry's mishandling of the recent and still-current scare about the infection of eggs by Salmonella bacteria might have been avoided if the once-luxuriant agricultural research establishment were still in being.) Similarly, while British researchers may rightly regret the numbers of erstwhile colleagues who have gone for good elsewhere, there is probably no way now of bringing them back.

Damage

The much more serious and lasting damage is that done in people's minds. And even if researchers may now believe that there are smooth waters ahead, the minds of others will retain the impression of a research profession under threat for a long time to come. That this must be so is shown by the still laggard recruitment of young people to science courses at universities and polytechnics, and by the shortage of graduates clamouring for careers in research.

That is why the British research enterprise must from now on pay more attention to its public reputation, especially among young people. Talking (and writing) about the excitements of research will help. So too will schemes such as those growing up between British universities and polytechnics with neighbouring groups of industries and schools. Although primarily intended to take the edge off recruitment problems, such devices may serve the broader purpose of demonstrating at first hand that research remains self-confident. But in the long run, what will matter more is that the research profession should be recognized as a successful one, either the matrix from which successful entrepreneurs spring or as one that enjoys the esteem of comparable professions, which means that among other things its members should be reasonably well-paid. There are some who remark regretfully that much of the government's extra £95 million this year will go on pay increases, but is that an unmitigated misfortune? Indeed, with the prospect of a true European Common Market no further away than 1992, should not the managers of the enterprise be planning to pay British scientists mainland salaries by then?

A further change that should not be forgotten is that worked by the pressures of the past decade on the temper of the grant-making agencies in Britain. The importance of the change is evident in the corporate plan (a name which is itself a pointer) of the Science and Engineering Research Council (SERC). Not so long ago, the council would have insisted in such a document that its function is to support research at universities; the range of laboratories it manages were all originally created to provide services (mostly equipment) that universities and polytechnics culd not manage on their own. But SERC tells how, "in association with HEIs" (presumably an acronym for Higher Education Institutions), it will "continue ... to support excellence in ... science and engineering". Its declared aim is that the ratio of resources spent on "strategic" and "basic" research will be in the ratio 60:40, identifying the former with "wealth-creation" and letting the reader guess that the latter has no economic significance, except perhaps in giving young people skills that may be put to economic use.

The shift of emphasis from the support of research institutions to the support of research itself is significant, and worrying. SERC's own spending on research grants has increased in the past few years commendably, but what has happened to the old notion that researchers, not committees, are the best (and most imaginative judges) of what might be done next? The distinction between strategic and basic research, like all attempts meaningfully to partition a spectrum by drawing a line across it, similarly makes no sense. As the whole world knows, it is not possible to tell with any certainty which research projects will create wealth and which will be largely academic in their consequences. But if there is some substantial parcel of British research which is certain to produce wealth, it would do so more even more effectively if it were market-orientated. In other words, SERC's policy is an invitation to some future government to complain that 60 per cent of what it does would be better hived off to industry. By insisting that these simple judgements of SERC's are heresy of a kind, the scientific community in Britain will not merely assure the integrity and thus safety of research, but may even help to save SERC from itself. □

Soviet nuclear dissent

Chernobyl is not the only reason why Soviet civil nuclear power is behind target. Can it pull back?

With the possible exception of the government of France, the Soviet government has so far been among the most resolute (and successful) in the pursuit of civil nuclear power. It is natural that the accident at Chernobyl should have given the nuclear planners pause, but that is not the only reason why 28 GW of planned capacity has been stripped from the original plans during the past year (see page 298). Even before the appointment of Mr Mikhail Gorbachev as Party general-secretary, there had been protests in republics as different as those in the south and the north-west at plans to build nuclear power stations there, often apparently as if to canalize more general discontent in directions that could not be held to be illegitimate. The coming of *glasnost* has inevitably complicated the planners' lives, creating for Soviet agencies problems to which they are unused — those of winning advance public approval for projects that, in the old days, would have been decided on strictly administrative grounds.

Now they are in a real fix. Members of the Soviet public have been given both a foundation (the Chernobyl accident, and the hair-raising evidence of mismanagement that it revealed) and a mechanism (*glasnost*, which is not merely a licence for the press, but for local and regional Soviets as well) for dissent. The result is that opposition to the nuclear power programme is no longer simply confined to what are called intellectuals, or to republic nationalists (the Lithuanian government last year declined to contribute, as required, towards the cost of a fourth reactor being built at a site on its territory), but is much more broadly based. Moreover, local and regional Soviets, whose consent is formally required for developments in their spheres of influence, can now speak up and have an incentive to do so. (Self-interest will have their members calculating that unpopularity with constituents may lead to defeat in some future popular election, if the electoral system does indeed develop along democratic lines.) Worse still, the nuclear planners find that the Soviet press has emerged into *glasnost* with a distinctly anti-nuclear predilection, born partly of Chernobyl and partly of the appearance of complacency among the managers. The result is that an article by somebody in high authority in a sufficiently august newspaper will no longer suffice to turn public opinion around. Nuclear planners in the West would find themselves on familiar ground in the Soviet Union now.

What is to be done? The Soviet target of 100 GW of nuclear generating capacity by the year 2010 looks unattainable. There are optimists who believe that 80 GW might be managed, but even that target must depend crucially on what happens in the next few years. Yet on traditional planning assumptions, nuclear generating capacity on some such scale will be needed if intended industrial developments are to succeed. Moreover, most of this extra power will be needed in the western regions of the Soviet Union, which happen also to be where most people live, and are now able to dissent. The assumptions on which the planners' forecasts are based may, of course, be incorrect, but in the absence of market pricing for different kinds of fuel, there is no way of being sure. Similarly, in the absence of direct incentives for users (as distinct from planners) to economize in the use of energy, the Soviet Union cannot easily expect that fuel efficiency will take the edge off the growth of electricity demand, as it has done in the West since 1973.

So there is no choice but to win round public opinion — a daunting task requiring that the Soviet nuclear industry should be even more adventurous in its exploitation of *glasnost* than even the most adventurous of the Moscow newspapers. Experience elsewhere (Britain, for example) shows that people must be told of routine mishaps at nuclear plants (as in industrial enterprises of all kinds) in the hope that they may eventually become familiar with the notion that the nuclear industry does not need to be literally free from accidents (but it has to be something of a paragon) to be safely manageable. It will be interesting, and may be instructive for the West, to see how brave the Soviet planners will be in these unfamiliar circumstances. But that they have little choice is beyond dispute. Meanwhile, in the search for nuclear generation sites, there seems little choice but to build new reactors at existing sites until the winds of dissent have abated. □

US budget pragmatism

The US budget problem is not its size, but how to cut it.

SENATOR Lloyd Benson's celebrated gibe at now-Vice-President Daniel Quayle, "You're no Jack Kennedy", might as well have been levelled at President George Bush last Friday, after his low-key inaugural address. But Bush is none the worse for that. Not every US president needs to sound like a Lincoln, a Roosevelt or a Kennedy, nor is rhetoric at the beginning of a four- or eight-year stint a guide to performance in the future. It may even help in present circumstances if the new presidency is seen to be manned by down-to-earthers who prefer to deal with real problems by solving them.

As yet, unfortunately, there are only straws in the wind as guides, and they seem to be blowing in contradictory directions. Everybody appreciates that US government support for research, like all its other discretionary expenditures, will be determined by the outcome of this year's tussle over the budget. As always, nobody will forget that this year's new congressmen (there are not many) will be campaigning for re-election before the next financial year (called fiscal 1990) is up. The best hope is that revenues can be modestly increased (preferably by natural growth, otherwise by devices that cannot this year be called taxes) and that expenditure can be selectively reduced. There are some signs of grace. The new National Security Adviser, the formidably phlegmatic General Brent Scowcroft, may have been less than enthusiastic about the Strategic Defense Initiative on television at the weekend: does that mean that a large part of $5,600 million, three times the budget of the National Science Foundation, has been saved already?

On the other side of the coin, there is a general air about the new administration suggesting scepticism of President Ronald Reagan's commitments to the arms control processes begun in the past six years. It is true, of course, that the new president is much less sceptical than was Reagan on entering office, and that it is natural that a new administration should turn a quizzical eye on the deals its predecessors have been making with others, if only to be sure of understanding them. It would not be the end of the world if the Bush administration should conclude, for example, that it would prefer to abandon the proposed 50 per cent reduction of strategic arms in favour of some other accommodation with the Soviet Union and its allies in the Warsaw pact.

Yet those in Washington now musing aloud that Mr Gorbachev is interested in arms control and other accommodations only because of his domestic economic problems (and Gorbachev appears to admit that his budget is part of his motive) should reflect that the same calculation could apply to the United States. The most inflexible part of the US budget is that (nearly a third) spent by the Pentagon. The years immediately ahead will be especially difficult because development projects will be clamouring for procurement. Would it not be a prize worth having to be able to cut the defence budget without endangering security? The new administration is only a few days old, and there are many urgent matters clamouring for attention, but it will be a misfortune of this trade-off has not arisen naturally before the budget negotiations reach their climax later in the year. □

Sakharov defeated by "odd arithmetic"

- Absentees assumed to vote against
- Academy relinquishes five seats
- Sakharov nominated for Moscow City

Moscow

THE failure last week of the enlarged plenum of the Soviet Academy of Sciences to nominate Andrei Sakharov for the forthcoming election of Soviet deputies has caused great surprise and raises the question whether Soviet people, including men and women of science, yet know enough about democracy to practise it.

Sakharov, who had been supported by 60 of the academy's institutes, was not the only disappointed candidate. There were also Roald Sagdeev (supported by more than 25 institutes), Dmitri Likhachev (20 institutes) and such prominent economists (active advocates of *perestroika*) as Gavriil Popov (15 institutes plus), Nikolai Shmelev (more than 12), Gennadi Lisichkin and Abel Aganbegyan.

Last week's proceedings were an intermediate stage in the election of the 2,250 members of the Congress of People's Deputies, which will meet once a year to decide constitutional matters and will also elect the bi-cameral Supreme Soviet. A total of 75 seats in the chamber has been reserved for representatives of professional associations and societies, of which the academy had 25. But scientists can be elected by other categories of voters. Thus Guri Marchuk, president of the academy, and Yevgeni Velikhov, vice-president, have been nominated as candidates by the Communist Party.

The 17-member electoral commission took two hours to determine the outcome of the voting, by secret ballot. When the results were announced 10 hours after the meeting had begun, those left on the platform and in the hall were equally surprised. The outcome was so unexpected that it seemed like the dénouement of a whodunit.

One strange consequence is that there are now fewer contenders (23) than there were originally vacancies (25) in the academy's intended representation in the Congress of People's Deputies. It had been planned that there would be a meeting in two months at which the contenders would compete, promoting their own platforms. But the first outcome of the plenum would have made meaningless the continuation of the election of the academy's list: the contenders would have had no rivals.

Accordingly, the members of the plenum decided to cut the academy's representation first by two and then by five, a decision endorsed by the praesidium (governing council). The spare seats will be filled by other academic societies and associations.

This development points both to the imperfections of the electoral machinery and to the likelihood that people do not fully appreciate their role in the democratic process.

This election is in two stages. In the first, nominations were made by the members of academy institutes and establishments. People at these meetings participated enthusiastically. Eventually there were more than 130 names on the list of those seeking the academy's nomination. Last week's plenum was intended to prune the list for a second stage at which choices would be made by all members of the academy and institute representatives (one for every 150 workers).

By prior agreement, the plenum did not discuss all the candidates because of lack of time. But it was agreed that the opinions of nominating institutes should be studied carefully, which is significant because the plenum thereby learned of the nominees' scientific and, more importantly, civic qualities. But the outcome of the plenum's procedural discussion was also to ensure a vote 'against' from each of its absent members.

Strictly, this accords with Soviet electoral law, which requires that those elected should receive "the vote of more than half the aggregate number" of those entitled to vote. Last week, 379 people were eligible to attend the enlarged plenum, but 102 of them were absent for various reasons. Whether the academy's election commission ever discussed with the central electoral commission the question of how the absent votes should be dealt with is unknown.

In the event, the qualifying quota was set at 139 votes, but only 190 people took part in the voting, so that the votes of the 89 people who did not were counted as 'against' in each case. Thus each contender had to secure 73 per cent of the votes to be elected. (Yablokov needed only one extra vote, but Sagdeev needed 18 and Sakharov 34.)

Much the same happened at the All-Union Society of Inventors and Innovators, where the same odd arithmetic meant that only six contenders instead of ten managed to secure qualifying votes. But that council reconvened the plenum to fill the 'vacancies'. Should not the academy have done the same?

One interpretation of the purpose of the academy's plenum is that it was to ensure that the final choice of its 25 representatives would be genuinely competitive, reflecting the will of the scientific community at large. This might reasonably have been done in keeping with certain criteria, such as the number of institutes backing each candidate.

Many of those not nominated had solid backing. It goes without saying that Sakharov was the undisputed leader in the race he eventually lost. But Sakharov is extremely popular outside as well as within the scientific community, with the result that he was nominated, at a meeting on 20 January at the Lebedev Institute where he works, as a candidate for people's deputy from Moscow's Octyabrsky district and, two days later, as a candidate in the election of a deputy to represent the City of Moscow. **Yuri Kanin**

Novosti

Tajikstan struck by earthquake

London

THIS week's earthquake in the Soviet republic of Tajikstan, although not as serious as the Armenian catastrophe last month, has inevitably added to growing concern in Tajikstan about seismic safety.

The earthquake is the third in four years. There was a major earthquake in the north of the republic in October 1985 and the collapse of a dam at Sargazan in March 1987. Land and mudslides, such as that which this week buried two villages, are a common accompaniment.

What concerns the population most is the rapid development of hydroelectricity in the republic. A further eight dams are now planned on the River Vaksh, which already has the massive Nurek (305 m) and Rogun (345 m) dams. People fear that earthquakes may rupture the dams, and even that the dams themselves may trigger earthquakes.

At a meeting two years ago in the Tajikstan capital of Dushanbe, representatives of the Tajikstan Academy of Sciences revealed that the dams had been designed for seismic conditions appropriate to a plain, not a mountainous region, where the effects of seismic disturbances are usually greater. *Pravda* described the new Ziddin reservoir as "millions of cubic metres of water hanging like the sword of Damocles" over Dushanbe.

Tajiks themselves, who say they have little need of the electricity which is mostly exported to Afghanistan and other republics, seem in little doubt. A few days after the Spitak disaster, environmentalists and others formally petitioned for a halt to dam construction. **Vera Rich**

NIH gives the go-ahead on genetic experiment

The controversial first experiment to involve the insertion of foreign genes into humans — which has been deliberated by seven US review committees and government agencies over the past several months — received approval to proceed last week. James Wyngaarden, director of the US National Institutes of Health (NIH), authorized Steven A. Rosenberg and W. French Anderson to readminister interleukin-2-stimulated T-lymphocytes marked with a gene for neomycin resistance to ten cancer patients from whom the cells were isolated. The inserted gene will provide no therapeutic benefit to the patients, but will enable Rosenberg and Anderson to determine if the cells return to the tumour to fight the cancer. C.E.

Vet schools to close

The University Grants Committee announced last week that two of Britain's six veterinary schools are to close. A working party appointed to consider the rationalization of veterinary education said that no school is at present able to teach clinical science with the necessary spread of disciplines and depth of coverage.

The schools at the Universities of Glasgow and Edinburgh will be merged to form a new school based in Edinburgh, and veterinary deg-rees will no longer be obtainable at the University of Cambridge. The last intake of students will be for the academic year 1989–90. The remaining schools, at the Universities of Bristol, Liverpool, London and Edinburgh, will have no fewer than 36 senior clinical teachers and will teach about 76 students each year. C. McG.

Scientists for Phobos

Ten US scientists, including Bruce Murray of the California Institute of Technology and Thomas Duxbury of the Jet Propulsion Laboratory, have been selected to participate in the Soviet Phobos mission, due to reach Mars and its satellite by April this year. The ten were chosen jointly by the National Aeronautics and Space Administration (NASA) and Soviet delegations to a US – Soviet working group — the working group was set up under the 1987 cooperative agreement on space research between the two countries.

Meanwhile a new agreement to foster scientific cooperation was signed in Paris earlier this month by outgoing US Secretary of State George Schultz and Soviet Foreign Minister Eduard Shevardnadze. In eight general areas, including science policy and problems of the Arctic as well as all the traditional disciplines, US agencies and their Soviet counterparts will draw up plans to facilitate cooperative research. D. L.

More criticism for NIH over 'summary' cut of grants

Boston

THE US National Institutes of Health (NIH) are drawing more fire for their decision to end financial support for two scientists accused of misconduct before an investigation into their case had been completed.

NIH procedures for investigating misconduct have recently come under heavy criticism. The current case involves two scientists at the Harvard-affiliated Massachusetts Eye and Ear Infirmary. Kenneth Kenyon and Scheffer Tseng (Tseng is now a researcher at the University of Miami) are alleged to have had business interests in an ophthalmic ointment they were testing with funds from NIH and to have delayed publication of results showing the ointment to be ineffective (see *Nature* **336**, 506; 8 December 1988). NIH suspended their research grants late last year, but are still investigating the affair.

But Claes Dohlman, outgoing chair of the ophthalmology department, this week sent a letter to NIH officials expressing his concern over the agency's action and protesting at what he calls the "summary way" in which NIH reached their decision. Dohlman's letter follows a similar one earlier this month from Charles Schepens and Richard Pharo, respectively president and director of research administration at the Eye Research Institute in Boston.

"To take action in the absence of evidence is unjustifiable", write Schepens and Pharo, adding that NIH's move sends a message to the medical research community that "the federal government is prepared to abandon a scientist without due process". Last month, Representative John Dingell (Democrat, Michigan), chairman of the House of Representatives Energy and Commerce Committee oversight and investigations subcommittee, which has been holding hearings on how scientific misconduct charges are investigated, wrote to Otis Bowen, the Health and Human Services Secretary, with similar complaints about the way the case was handled (see *Nature* **336**, 706; 22 December 1988). Dingell, in a sharply worded letter, accused NIH of reaching their decision solely on the basis of newspaper reports and called upon NIH officials to explain the rationale and precedent for their decision.

In a letter responding to Dingell's concerns, Robert Windom, Assistant Secretary for Health of the Department of Health and Human Services, writes that NIH "acted responsibly" on the basis of the information they had at the time, which he states was "sufficient to demonstrate a clear, urgent need for interim administrative action". Windom claims that, in addition to newspaper accounts, the agency based its decision to terminate Kenyon's and Tseng's financial support upon conversations with Harvard officials and upon a statement written to the Harvard faculty about the case by Daniel C. Tosteson, dean of Harvard Medical School. Jack McLaughlin, associate director of the National Eye Institute of NIH, did acknowledge, however, that the agency first learned about the case through newspaper accounts.

Both Windom and McLaughlin assert that there are precedents for NIH's move, although neither points to any specific case. McLaughlin stresses the importance of treating such incidents on "a case-by-case basis", and says only that he is sure that similar "interim actions" have been taken in other cases at NIH.

A staff member on Dingell's subcommittee has called NIH's response "disingenuous" and "inconsistent" with the subcommittee's records of what transpired in NIH's decision. Hearings on the matter by Dingell's subcommittee are expected to begin in early March, and reports are due before that from at least one of NIH's investigations. **Seth Shulman**

Vice-chancellors' pay offer to staff rejected

London

UK university management last week tried temporarily to resolve the pay dispute with staff by offering to increase salaries by 3 per cent from April 1989 and make a lump sum payment at that time equal to a 3 per cent rise for the period from 1 January to 31 March 1989.

After making the offer, the Committee of Vice-Chancellors and Principals acknowledged that it was too low. Sir Mark Richmond, chairman of the committee, said it was embarrassed at the inadequacy of the offer. There was a desperate need to pay academic staff decent salaries, but the committee thought staff would rather be assured of something now while it continued to argue for more. The vice-chancellors are to approach the government for extra money for 1989–90 which would be used by individual universities to reward merit and make discretionary payments to teachers in subjects short of staff.

The Association of University Teachers rejected the offer and will continue its boycott of university examinations. But it was cheered by the vice-chancellors' offer to backdate the offer to cover part of 1988–89. Christine McGourty

Guidelines produced for the use of transgenic animals in research

London

With transgenic animals finding increasing favour in both biomedical research and biotechnology, the UK Advisory Committee on Genetic Manipulation (ACGM) has produced detailed guidelines on their use. The guidelines, which the committee hopes will set an example for other countries, are particularly concerned with the consequences of the deliberate or accidental release of transgenic animals into the wild, and the hazards of using viral, and especially retroviral, vectors for transgenes — that is, the use of genetically modified viruses to introduce new genes into animals.

ACGM, which will need advance notification of any plans to use viral vectors or to release transgenic animals, warns that it will be tough on both. For animals that are to be released, there will be a ban on the use of any viral vector that contains even a partially functional oncogene and on any form of vector that has even a remote potential to replicate. Plans to use equivalent vectors to produce contained transgenic animals will be considered individually.

Among the considerations will be the precautions that have been planned to ensure containment. For large animals, ACGM takes the view that a well-fenced field is sufficient. Extra care will be needed for fish and other aquatic vertebrates because of the relative ease with which they or their gametes can escape. Even greater precautions are necessary for work with invertebrates that "crawl, jump or fly".

Some of the guidelines on genetic manipulation anticipate changes in the official Health and Safety Regulations, which were drawn up in 1978. Revised regulations suggested by the Health and Safety Commission in October 1987 have since been modified, and now await approval by the Secretary of State and parliament. Their main intent is to establish a legally backed compulsion both to notify ACGM of the production and use of transgenic animals and to set up and operate local risk assessment committees at any establishment that carries out genetic manipulation.

ACGM's guidelines also cover aspects of work with transgenic animals that fall outside its own purview. The welfare of such animals is "dealt with adequately by existing legislation", the guidelines say, pointing out that breeding from transgenic animals is regulated as though an animal experiment until it can be demonstrated that the progeny are not likely to suffer any adverse effects.

The guidelines advise that the consumption of transgenic animals or their products by humans or animals would be subject to consideration by the Advisory Committee on Novel Foods and Processes, which may in turn seek advice from ACGM. Professor Mark Williamson, who chaired the working group that drew up the new guidelines, says it is inconceivable that approval would be given to the consumption of animals containing a human transgene. **Peter Newmark**

HIV a problem for Indian blood banks

New Delhi

India is suffering from a wave of public alarm caused by news that human immunodeficiency virus (HIV) is increasingly being transmitted by use of donated blood. This development conflicts with the earlier belief that AIDS is being spread chiefly by prostitutes and foreigners.

Officials say that the government can no longer postpone compulsory screening of all blood banks, a suggestion that only six months ago was dismissed as unnecessary as well as impracticable.

No transfusion-related HIV infection had been detected in India until July 1987, which is one reason the government was content with only minimal restrictions on imported blood products. But since then, the proportion of seropositive cases ascribed to transfusion has been increasing rapidly.

Last month alone, the All-India Institute of Medical Sciences in New Delhi discovered antibodies to HIV in three haemophiliac patients, and doctors in another hospital said that 10 of their 6,136 voluntary blood donors tested positive for AIDS. Of the 620 confirmed cases of HIV infection in India, about 60 have received blood transfusions in Indian hospitals.

According to the Indian Council of Medical Research (ICMR), which operates some 40 AIDS surveillance centres, blood transfusion has become an important mode of HIV transmission. An estimated 1.5 million units of blood are used each year in India, but ICMR's centres can perform only 30,000 tests, using kits imported from Britain. Complete blood screening would require a 100-fold increase in the number of kits. Although ICMR feels that all donated blood must be screened, the job "cannot be done overnight".

As a first step, all donors in Bombay and Madras are now being screened, and experience from these cities will be used to start screening in other cities in about a year, by which time India hopes to be producing its own test kit. K.S. Jayaraman

Italian environmental programme launched

Turin

The Italian government last week launched a £70 million programme of research into environmentally benign technology. Research priorities must be formulated within two months by a new commission which will report to the ministries for industry and for the environment which are financing the programme.

The announcement was well-timed, coinciding with an international conference in Turin designed to increase public awareness of the problems of the greenhouse effect, the destruction of the ozone layer and acid rain and to stimulate political action to tackle these problems. Among the recommendations of the conference was a proposal to impose a tax on fossil fuels which would finance a world fund to promote alternative energies and energy efficiency. The conference also recommended improved monitoring of climate change and increasing research into an understanding of the problems. The Italian government was heavily criticized for the lack of support for such research. Money is scarce and research uncoordinated, as well as being hindered by a lack of access to meteorological data, which is controlled by the defence ministry. Christine McGourty

Testing of *in vitro* embryos approved

Melbourne

An Australian parliamentary committee has approved a controversial request by scientists at the Monash Medical Centre in Melbourne to test human embryos obtained for *in vitro* fertilization (IVF) for birth defects before implanting them in patients.

Tests will be done about two days after fertilization, when the embryo has grown to four cells. One cell will be removed and analysed for genetic defects. The tests will be performed only on embryos that take longer than usual to fertilize. According to Monash Medical Centre's Professor Carl Wood, the Australian pioneer of IVF, about 3 per cent of embryos have a delayed fertilization, but are not neccessarily abnormal. The tests will prevent normal embryos from being discarded. Embryo biopsy does not seem to damage the survival chances of the remaining embryo.

The decision of the committee, chaired by Professor Louis Waller of Monash University, may cause problems for Victoria Health Minister David White, who has written publicly that there will be no experimentation on human embryos older than 22 hours. The scheme may also face legal problems, as it involves the use of fertilized embryos but is not directly related to alleviating infertility. Tania Ewing

UK research grants suffer from SERC's lack of money

London

FIGURES published this week in the Science and Engineering Research Council's corporate plan highlight the growing imbalance in supply and demand for UK research grants. Expenditure has increased slightly since the first plan was published in 1984–85, but costs have increased further and the council cannot support the increasing number of grant applications. The research grant success rate for alpha-rated proposals decreased from 72 per cent to 48 per cent in science and from 98 per cent to 86 per cent in engineering. For standard grants, the success rate decreased from 28 per cent to 20 per cent in science and 39 per cent to 32 per cent in engineering. The council hopes to increase the percentage of grant-in-aid spent on research grants from 33 per cent in 1987–88 to 40 per cent, and to maintain at a constant level the numbers of research assistants supported by its grants.

Expenditure on equipment must increase, and the council hopes to achieve this by increasing the proportion of grant money that supports equipment and by allocating for equipment money for the interdisciplinary research centres (IRCs). Five IRCs have been established and the council plans for another 17 by 1991. More than 300 applications to host the centres have been received. But the plans for expansion depend on the money available. Only 50 per cent of the cost can be met from existing funds, says the council. It will seek further money from the research council's flexibility fund and from the Department of Education and Science.

The council plans to continue increasing the proportion of its budget devoted to strategic research with the aim of achieving a 60:40 ratio between support for strategic and for basic research by 1990–91. In the past decade, support for strategic research has increased from 42 per cent of total expenditure in 1978–79 to 54 per cent in 1987–88. For basic research, the council would like to make the support mechanism more long-term and to link expenditure to the country's gross domestic product.

Other council plans include an increase in expenditure on the LINK programmes, which are supported jointly by the research councils, government departments and industry. It plans to initiate eight more programmes annually over the next three years and to increase annual expenditure to £20 million in five years.

The council is also planning a collaborative programme of atmospheric research with the Natural Environment Research Council, to integrate satellite, ocean, Earth and atmosphere measurements with gas-reaction kinetic data and with related computer simulations.

Expenditure on space science will not be increased and, so that it might in future select parts of the science programme of the European Space agency in which to get involved, the council urges a review of ESA's method of operation to include some optional elements.

Christine McGourty

Soviet bloc research criticized

London

COMECON'S Comprehensive Programme of Scientific and Technological Cooperation, the first major policy development of the Gorbachev era, has been roundly criticized on Prague radio. Comecon, the trade and development alliance of the Soviet bloc, is celebrating its fortieth anniversary this week.

The programme was intended, when launched in December 1985, to encourage joint enterprises between member countries and was regarded as a step towards decentralization. Previous policy had been heavily orientated towards the Soviet Union, with a Soviet "head" institute for every project. The best-known exception had been the low-temperature magnetism laboratory at Wroclaw in Poland.

Radio Prague commentator Vladimir Vercak now says that within a few months of the adoption of the new programme, it was clear that differences of economic management in member countries would prevent the goals of the programme being attained. Joint enterprises have been successfully established only where long-standing partnerships already existed, but the lack of an intra-bloc currency is also an impediment. Unless the economic conditions of member states are rapidly improved, he concludes, the programme will not realize "Comecon's scientific and technological potential".

A NATO conference last autumn showed that there may be other problems. Speakers with first-hand knowledge of bloc countries drew attention to the way that the lack of equipment and resources tempts scientists and engineers to seek collaboration outside the bloc, while enterprises are often more interested in preserving their chances in world markets than in the needs of its socialist partners.

Vera Rich

Most French pupils to go to university

Paris

A major overhaul of the French education system published last week by Education Minister Lionel Jospin aims to make France the best-educated nation in Europe by the year 2000. The plans, estimated to cost between $8,000 million and $160,000 million, include massive recruitment of teachers, better teacher training and a revised curriculum. The goal is that 80 per cent of school-leavers should be able to go to university, while the rest should not leave school without a diploma in business or industrial skills.

Education and research were priorities in President François Mitterrand's pre-electoral "letter to the nation" last spring. Despite a national curriculum, with 90 per cent of pupils attending state schools, only 40 per cent get the chance of a university education. Every year, 100,000 pupils leave school without any qualifications. Over one third of school leavers who do obtain their *'bac'* (university entrance certificate) and go on to university fail to complete their course, many dropping out after the first year having failed the stiff examinations.

Jospin says that schools have an unwritten contract with children and their parents to provide the education they desire. One necessary step needed to fulfil this contract is to reduce class sizes. To this end, 300,000 new teachers are to be recruited over the next ten years. To make the profession more attractive, money will be provided to close the gap between the university-oriented 'lycées', and the less specialized, but also lower status, 'colleges', which are understaffed and have a high failure rate at the *baccalaureat*. Teacher-training institutes are to be set up and secondments introduced to allow teachers to improve their career prospects, as well as to catch up in their subjects.

The two main concerns with Jospin's plans are that success rates will be made artificially high by lowering pass marks, and that already overcrowded and crumbling universities could not cope with a large influx of students. Despite these objections, and some resistance from lycée teachers, the reforms have met with general approval.

Peter Coles

Correction

The high field test apparatus SULTAN at the Paul Scherrer Institute (see photograph in *Nature* **336**, 338; 1988) consists of magnetic spools contributed by ENEA and ECN. The design of the facility and the cryogenic infrastructure was realized by the Paul Scherrer Institute. Euratom helped to finance both construction and running costs of the apparatus.

AAAS annual meeting draws largest crowd of decade

San Francisco

THE annual meeting of the American Association for the Advancement of Science (AAAS) in San Francisco drew nearly 10,000 scientists, journalists and members of the public, making it the largest, and according to its organizers, the most successful meeting of the decade.

But the annual meeting still lacks a clear identity, and efforts to move it closer to a mainstream scientific meeting were not an unqualified success.

Since a low of only 2,300 people attending the Los Angeles meeting in 1985, AAAS has worked hard to boost attendance at its meetings. One approach that outgoing AAAS president Walter Massey credits with attracting a broader audience to the meetings has been special scientific symposia. These symposia, requiring a separate registration fee, were first held three years ago. This year's symposia on plant molecular genetics and protein folding drew standing-room-only crowds in 500-seat ballrooms.

But in neighbouring rooms of equal size, scientific symposia covering similar basic science topics seemed empty by comparison. Twelve sessions over four days brought top scientists from around the country to San Francisco for sessions on cellular receptors, developmental biology and gene expression and retroviruses and oncogenes, but audiences were disappointingly small.

Corey Goodman, professor of biochemistry at the University of California at Berkeley, who co-organized the developmental biology session, says he was mortified to find only 60 or 70 people in a hall that holds 500 listening to a paper presented by Harvard's Philip Leder.

Goodman maintains that a similar talk would have drawn hundreds at any of the San Francisco area's research universities. Each of the scientific sessions had several scientific luminaries who would typically draw large crowds, but none was well attended.

Goodman also complains that after exhorting him to coax the best scientists to come to the meeting, often at their own expense, AAAS then did little or nothing to promote attendance at the sessions. Goodman says that others who were asked to organize special symposia were similarly disappointed by the results.

Massey says he is not sure why these sessions failed to attract larger audiences, and the problem will have to be sorted out before the next meeting.

The San Francisco meeting did have its own social issues. A polite demonstration by an AIDS activist group disrupted an awards ceremony for AIDS researcher Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases.

Outside the meeting, animal rights activists distributed leaflets and held posters protesting at the use of animals for scientific research. The AIDS protesters threw confetti as part of their protest against the red tape involved in approving new AIDS drugs, said Massey, a stark con rast to the 1971 meeting in Philadelphia at which protesters threw blood in an anti-war protest.

The most controversial session of the current meeting occurred on the last day, when J. Philippe Rushton of the University of Western Ontario presented a paper using anatomical, genetic and behavioural studies as a basis for claiming that blacks exhibit less sexual restraint, have lower intelligence and have more violent temperaments than Caucasians or Asians. When news of the Rushton talk began circulating among the 771 journalists registered for the meeting, Massey was brought to a hurriedly scheduled press conference to deny that AAAS supported Rushton's theories, but to emphasize that it supported his right to express his opinion.

As an organization, AAAS is in the midst of change. In addition to the annual meeting, AAAS's most visible role is as publisher of *Science*. But the organization also has active programmes in public understanding of science, arms control and human rights. From 1975 until 1987, William Carey served as AAAS executive director, and with an insider's background in the workings of Washington, Carey put AAAS in the thick of the US science policy debates.

Carey was replaced by Alvin Trivelpiece, who had previously been an assistant secretary at the Department of Energy where he was widely credited with convincing President Reagan that he should approve plans to build the Super-conducting Super Collider.

But Trivelpiece resigned late last year to become director of the Department of Energy's Oak Ridge National Laboratory. His leadership was too short to establish any clear direction for the organization. Former *Science* editor Philip Abelson is acting executive director while the search for a successor proceeds.

AAAS has embarked on a series of long-term reviews on where it has been and where it is going. Massey is confident that the annual meetings will regain much of the vitality they had in the 1960s and early 1970s, when AAAS became a focus for the intersection of science and society.

Joseph Palca

Nuclear plant presents unique problems

Boston

THE first attempt ever to decommission a commercial nuclear power plant reached a crucial milestone last month when the reactor vessel of the Shippingport Atomic Power Station was hoisted out of its underground site. The reactor's removal is perhaps the largest single step in the Energy Department's five-year, $98-million project to decommission the power plant, located outside Pittsburgh, Pennsylvania, but many steps still remain.

Built in 1957 as part of the "Atoms for Peace" programme, Shippingport was the world's first nuclear plant to operate solely for the production of electricity. It closed in 1982, and work on dismantling it has been going on for three years.

The 72-MW reactor was constructed in a joint venture between the now-defunct Atomic Energy Commission (AEC) and a private utility, and the government agreed at the time of its construction to take charge of its decommissioning.

All other US commercial reactors will have to be decommissioned by the utility companies that built them, focusing industry interest on the Shippingport project. In the United States, 15 plants are expected to be ready for decommissioning by the year 2000.

The Department of Energy (DoE) wants to use the Shippingport reactor as a model programme for decommissioning. But some critics say that experience gained at Shippingport will have limited relevance to future decommissioning, and will only emphasize the obstacles others will face.

The most glaring obstacle is that in the United States there is still no high-level nuclear waste repository for spent fuel rods from commercial reactors, and virtually no place to deposit low-level waste. All three of the existing low-level facilities are close to capacity and nearing the end of their designed life-spans. For commercial reactors now and in the foreseeable future, disposal of radioactive refuse from a decommissioned plant would be impossible.

But even beyond the disposal problem, critics say that DoE is not making as much of the exercise as it could for testing and evaluating techniques needed in the future. They also object to DoE's decision to remove the reactor vessel for disposal in its entirety rather than cutting it into pieces on site as will be necessary for larger plants. Even with the reactor vessel's removal, the job is far from over. On the Shippingport site, two more years of work are expected.

Seth Shulman

Soviet nuclear power plant programme marks time

Moscow

THE sad accident at Chernobyl three years ago has cast a shadow on the nuclear power programme of the Soviet Union. Although the Minister of Nuclear Power Engineering, Mr Nikolai Lukonin, has only recently reaffirmed the government's commitment to nuclear generation as a means of meeting growing electricity needs, planned nuclear developments are now being resisted vigorously by the public and by local authorities in some republics.

The decision to close the two reactors of the Armenian nuclear plant near Yerevan, after a reassessment of the seismic hazards following last month's earthquake, is a further aggravation of the problems. One reactor will be closed on 25 February, the second on 18 March, after the expected peak of winter electricity consumption. Electricity supply in the Georgian republic will afterwards be tightly constrained.

Growth of nuclear generation capacity has been practically zero over the past year, with 45 reactors at 16 sites with a total generating capacity of 34.4 GW. Total generation last year, at 215 GWh, was 12.7 per cent of electricity consumption. But although new capacity is under construction at 15 sites, the building programme has been substantially pruned. A total capacity of 28 GW has thus been lost.

Nuclear construction projects have been stopped in Azerbaijan, Georgia, Armenia, Lithuania, Byelorussia, the Ukraine and in the south of the Russian Federation. And it can be confidently presumed that further nuclear power stations will be vetoed.

These developments create an awkward predicament for the Ministry of Nuclear Power Engineering, formed after the Chernobyl accident with responsibility for eradicating the consequences of the accident. The ministry is retraining personnel at all nuclear plants, revising construction and operating standards and improving safety arrangements. Lukonin now acknowledges a need for greater openness, and an open dialogue with the public. Trust is easily lost, but regained only with difficulty.

The Soviet government's decision to invite the international Operational Safety Review Team (OSART) to the Soviet Union for three weeks in December is part of the process of regaining trust. The team, including staff members of the International Atomic Energy Agency (IAEA) and independent experts from a dozen countries, made a detailed examination of the 1,000-MW pressurized water reactor at the Rovno power station in the Ukraine.

The preliminary conclusions, given at a press conference in Moscow by Ferdinand Franzen and Morris Rosen, respectively head of the IAEA safety division and director of nuclear safety, are generally positive. The group acknowledged the competence of the operating staff, the priority given to safety (hardly surprising after Chernobyl) and that radiation shielding and ecological aspects of the plant's operation were up to international standards.

Among shortcomings at the Rovno plant, the group draws attention to the poor quality of some of the equipment arriving there (whose defects must be made good on site) and shortages of some modern instruments and automatic devices. The OSART team also plans to recommend in its final report that the station's senior executives should be given more decision-making power.

Lukonin says that his ministry is taking steps to toughen requirements for site selection, the supervision of construction and the acceptance of equipment, while the ministry responsible for instruments says that it is making good the shortages which have been noted. The recommendation that station managers should have more authority, meant to secure greater flexibility over decisions, would presumably also encourage them to act more responsibly: Chernobyl showed that the over-meticulous tutelage of the higher authorities breeds complacency.

The immediate effect of the OSART mission on Soviet public opinion will be to demonstrate the government's readiness to follow the highest international standards in the operation of nuclear plants. There have already been similar inspections of 29 reactors in 16 countries, presumably with similar effects. The next inspection in the Soviet Union will be of the two 500-MW heat-supply reactors being built at Gorky.

Yuri Kanin
Novosti

More turmoil at German universities

Munich

DESPITE government approval in December of an emergency aid programme for the overcrowded West German universities (see *Nature* 336, 704; 22/29 December 1988), demonstrations and strikes continued after the Christmas vacation.

The situation in Berlin, where the president of the Free University had to call for police help to keep open laboratories in the medical faculty, seems to be the most serious. Students there who were not on strike had complained that they were being deprived of their right to study. Most medical students have since returned to classes, but students in other faculties remain on strike, demanding more say in governing the university and an improvement in the abysmal conditions caused by overcrowding.

There have been demonstrations and strikes at other universities in West Germany. In Munich, more than 30,000 students from both the Technical University and the Ludwig-Maximilian-University marched peacefully through the middle of the town demanding more low-cost student housing, more professors, more student grants and loans and the construction of a long-discussed subway line to the campus at Garching.

All parties in the Bundestag (parliament) called on 18 January for "relief" of the overcrowding, but without being specific. West German Education Minister Jürgen Möllemann (Free Democrat) said he would try to persuade the cabinet to add DM400 million to the DM2,100 million emergency programme voted in December.

Steven Dickman

Stock Exchange tries to stop research leaks

London

LEAKING to the financial markets of information in medical journals before their publication is causing concern in the London Stock Exchange. Results in the *British Medical Journal* (BMJ) of a report favourable to Glaxo's Zantac were leaked the day before publication, causing the company's share price to increase unexpectedly and more than 3 million shares to be traded that day. An investigation of the incident was initiated by the Stock Exchange's surveillance unit; no evidence of insider dealing was found.

Since then, the Stock Exchange has been considering how such incidents might be avoided. One proposal is that drug companies be asked to inform the Stock Exchange if information about to be published is price-sensitive; notice of this would then be released on the Stock Exchange's news service before publication.

At present, pre-publication copies are issued to journalists; this information is often leaked to analysts when journalists ask for comment on new results. The BMJ this month warned journalists receiving advance copies that it will not continue to supply copies to journalists found to have disclosed any information before publication. Analysts would prefer to receive information at the same time as journalists, but that will not put an end to leaking. It is the analysts' job, said one, to find out what is not in the public domain and to use that information.

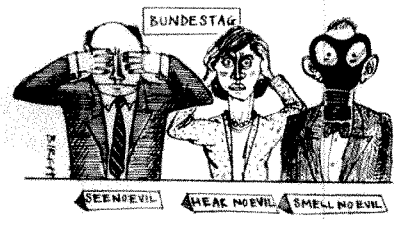
Christine McGourty

West Germany admits it knew of Libyan chemical plant

Paris

MORE evidence emerged last week that West German companies have been involved in the construction of an alleged chemical arms plant at Rabta, Libya, bringing a vigorous attack in the West German Bundestag (parliament) on 18 January of the federal government's handling of the affair.

Chancellor Helmut Kohl was accused of damaging West Germany's relationship with the United States, bringing shame upon the Bundestag, prolonging the threat of chemical arms and covering up the involvement of West German companies in their manufacture.



For seven months, Kohl's government had shrugged off increasingly noisy US claims that Libya was building a chemical arms factory, and that West German companies were involved (see *Nature* **337**, 199; 19 January 1989). But last week Chancellery chief Wolfgang Schäuble admitted to the Bundestag that both allegations were justified.

Schäuble confirmed that the government had been aware since May 1988 of the possible involvement of West German companies in building and supplying the factory. But he defended the government's silence, saying that customs investigations of one company, Imhausen-Chemie GmbH, had failed to produce sufficient evidence for a prosecution.

Last July, West German customs officials were warned by foreign intelligence agencies that a Frankfurt-based company, Ihsan Barbouti International (IBI), was suspected of engineering an illegal supply route to divert construction materials and chemicals to Libya. But it is only this year that documents seized from IBI offices have confirmed the clandestine operations. The company is now in liquidation.

IBI, set up by Iraqi businessman Ihsan Barbouti, used a Hong Kong company that was also linked with Imhausen-Chemie to obtain legitimate export licences. But cargoes that included architectural plans from Imhausen-Chemie and ventilation equipment and dichloroethane from two other German suppliers were diverted to Libya.

Legal action is now being taken against IBI for falsification of export documents. But it is still uncertain whether the suppliers, including Imhausen-Chemie, Merck and Alfred Tewes GmbH, actually broke the law.

Despite tighter controls, the volume of export trade — about 2.5 million shipments a month from West Germany — makes it impossible to halt illegal trading completely. Now other governments are looking to see if they can point an untainted finger at West Germany.

Peter Coles

Japan denies involvement in Libya

Tokyo

JAPAN's Foreign Ministry has firmly denied a US claim that two Japanese companies provided Libya with equipment for a chemical weapons plant. According to the spokesman, Yoshifumi Matsuda, the disputed "metallurgical complex" built in Libya by Japan Steel Works was designed to produce parts for a water purification plant, not shells for chemical weapons as had been claimed.

Allegations about Japan Steel Works and another company, Marubeni, hit the Japanese headlines last week after US Senator Jesse Helms included their names in a list of 36 companies, most of them West German, which he submitted to the Senate Foreign Relations Committee. All were alleged to have had links to the Libyan plant.

But Matsuda says that Senator Helms's list is based only on newspaper articles. In December, the *Christian Science Monitor* mentioned Japan Steel Works in an article on chemical warfare. The Ministry of International Trade and Industry (MITI) has since had time to investigate the allegations and found them to be untrue.

According to a MITI spokesman, a close watch is kept on products that might be used to build chemical weapons, and the export of nine key chemicals from Japan is prohibited without special licence.

Alun Anderson

AIDS test law

BULGARIA has introduced tough new legislation aimed at halting the spread of AIDS. Unlike other socialist countries, where anti-AIDS legislation concentrates on curbing the sexual activity of people known to be HIV-positive, the Bulgarian law concentrates on compulsory testing and treatment. Heavy fines will be imposed on those who refuse tests, and also "persons who do not fulfil their official duties in the fight against AIDS". V.R.

Another blow for creationism in California

Berkeley

THE California Board of Education has passed a policy statement on the teaching of science aimed at strengthening the message that evolution, not creationism, belongs in the science classroom. The statement supersedes a vague "antidogmatism policy" which often left teachers uncertain about how strongly they should stress evolution.

The antidogmatism statement, adopted in 1972 when Ronald Reagan was governor, was a brief directive on the discussion of "origins of life and earth", that required that "dogmatism be changed to conditional statements where speculation is offered as explanation for origins". California creationists, including members of the Institute for Creation Research in San Diego, have used the statement in their efforts to water down the teaching of evolution in California classrooms.

In 1981, a Superior Court judge decided against creationists in a case challenging the teaching of evolution as dogmatic. His decision affirmed that teaching of the scientific method is by definition not dogmatic, and that teaching of creation in public schools constitutes religious instruction, and is therefore constitutional.

But he upheld the antidogmatism policy as the ruler against which science education was to be measured. Scientists and teachers complained that the policy singled out the theory of evolution as somehow little more than conjecture, ignoring the true definition of a scientific theory and the fact that all science is based on theories that have stood the test of time. Many teachers, they said, felt compelled by the policy to couch their discussions of evolution in speculative terms.

The new policy statement carefully defines theories as tools of science, different from beliefs because they are logical constructs based on evidence, and subject to testing, modification and refutation. Beliefs, it implies, are closer to dogma, as they are based on ideology and not subject to test. A spokesman for the education board says that by requiring science teachers to teach only what falls within the definition of science, and directing administrators to support teachers in their resistance against pressure to do otherwise, the statement's drafters hope that it will provide firm support for science teachers who are put under pressure to teach creation in their classes.

The board is also planning to reject for use in California classrooms textbooks that do not deal adequately with evolution.

Marcia Barinaga

Lessons from eugenic history

SIR—Among the four papers on schizophrenia in the 10 November 1988 issue of *Nature*, there was one brief reference to an early work by the German psychiatric geneticist, Professor Ernst Rüdin[1,2]. Unfortunately, there was no discussion of Rüdin's work or his career, subjects painfully relevant to the topic of the inheritance of schizophrenia. Rüdin was an internationally recognized psychiatrist-geneticist whose field of interest was the genetics of schizophrenia. In 1928, he became director of the Kaiser-Wilhelm Institute of Psychiatry in Munich. Rüdin was one of the principal architects and advocates of the Nazi programme of enforced eugenic sterilization, which was an application of his work on the inheritance of psychiatric disorders. The programme saw the sterilization of about 50,000 German citizens a year, beginning in 1934. It established a 'scientific' basis for the mass control of reproduction with the objective of eliminating undesirable traits of human life that were considered to be hereditary[3]. Criteria for sterilization in Rüdin's institute included being a conscientious objector, a frame of mind that was considered to be a form of schizophrenia and consequently classified as hereditary[4].

Rüdin's genetic/eugenic fervour was not confined to compulsory sterilization. He was a supporter of the 1935 Nuremberg race laws, which he considered to have been an achievement for his eugenics movement[5]. He served as a member of a group of psychiatric 'expert witnesses' for the euthanasia programme of medicalized murder which, the 'experts' felt, would "meet with general understanding and approval, as it becomes established and more generally known that. . . all possible measures were taken either to cure the patients or to improve their state sufficiently to enable them to return to work which is economically worthwhile. . ."[6].

To the uninformed, psychiatry appears to be a naive 'Cinderella' specialty that is only now joining the ranks of experimental genetics. The reality is that psychiatry has been a wicked 'step-sister' in the field of experimental genetics, a nefarious role which Ernst Rüdin helped to define. What is new today is the sophistication of genetic research which enables science to explore the molecular basis of human heredity in a manner undreamed of in the days of Rüdin. What is still needed, however, is a process that ensures that human values are appropriately applied with a sensitivity and a sophistication that takes into consideration the recent history of science and medicine where values and science were distorted to the detriment of all mankind. By ignoring this history, science has demonstrated that it has yet to achieve the sensitivity required to pursue this type of research in a manner which is in the best interests of human society.

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Suffer the animals

SIR—If G.E. Lamming's account of an international symposium on biotechnology in growth regulation (*Nature* **336**, 19–20; 1988) is comprehensive, as I have no doubt it is, it implies that the subject of animal welfare was not considered seriously at that symposium. The absence of a session on the welfare aspects of genetic engineering reinforces the impression that little more than lip service is being given to the humane aspects of what is a headlong rush for quick profits by the meat-production industry.

It is clear from observations of transgenic animals so far produced that they frequently develop unexpected abnormalities that cause them considerable suffering. Indeed, Lamming mentions that transgenic pigs made by researchers at the US Department of Agriculture suffered high mortality and morbidity.

Only an elementary knowledge of molecular genetics is necessary to predict that the random insertion of foreign DNA into an animal's genome is likely to cause abnormalities in at least some of the recipients that will lead to real suffering. It is important that those involved in the production of transgenics should be seen to be taking particular care over this aspect of their work, for example by including it on the agenda of international meetings.

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Farewell Soil Bureau

SIR—As a result of an institutional rearrangement by the Department of Scientific and Industrial Research (DSIR), the New Zealand Soil Bureau has disappeared. After more than 50 years of carrying out soil research and providing soil maps, it has ceased to exist as an independent unit. Founded when the New Zealand economy depended almost entirely on the soil, it has succumbed t the new style of New Zealand life and th reforming zeal of the government.

Probably its greatest triumph was th detection of the lack of critical trac elements in volcanic soils, which allowe successful sheep farming over wid regions of the North Island; and mor recently the painstaking investigation c aspects of the Abbotsford landslid gained the admiration of the geotechnic community.

Soil science has now been incorporate into the new DSIR Division of Land an Soil Science, but the rationalization ha meant that more than 20 scientists hav lost their bureau jobs, including Benn Theng, Graham Claridge, Pauline McCo and Iain Campbell. Soil science in Ne Zealand has been diminished, as it has i the United Kingdom. It seems odd that a we become aware of major 'green' issues agencies directly involved with their inves tigation are closed.

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Credit where it's due

SIR—In their 'anniversarial' article (*Nature* **337**, 29; 1988) W. F. Bynum and J. L. Heilbron, discussing the cell theory of the German botanist Theodor Schwann (1810–82), say that the famous phrase *Omnis cellula e cellula* "came later". Thus, by inference, they sustain the widely held but erroneous attribution to Rudolf Virchow (1821–1902).

In fact, and in print, it was first used by François-Vincent Raspail (1794–1878), as epigraph for his thesis "Développement de la fécule dans les organes de la fructification des cereales" (1825)[1], in which he refers specifically to the origin of new cells by division. The anatomist Matthias Schleiden (1804–81), Schwann, and later Virchow all appear to have lifted one or more of Raspail's prior contributions to the cell theory without acknowledging their source. The nationalistic nature of their behaviour is epitomized by a ringing declaration made in 1866 by the surgeon Pierre-Paul Broca (1824–80), that "the cell is French and belongs to M. Raspail", (quoted by Wiener, 1968)[2]. But Broca was correct. It is time to remedy a gross injustice and to give Raspail, who is incidentally the undisputed founder of the science of histochemistry, his due recognition as father of the cell theory.

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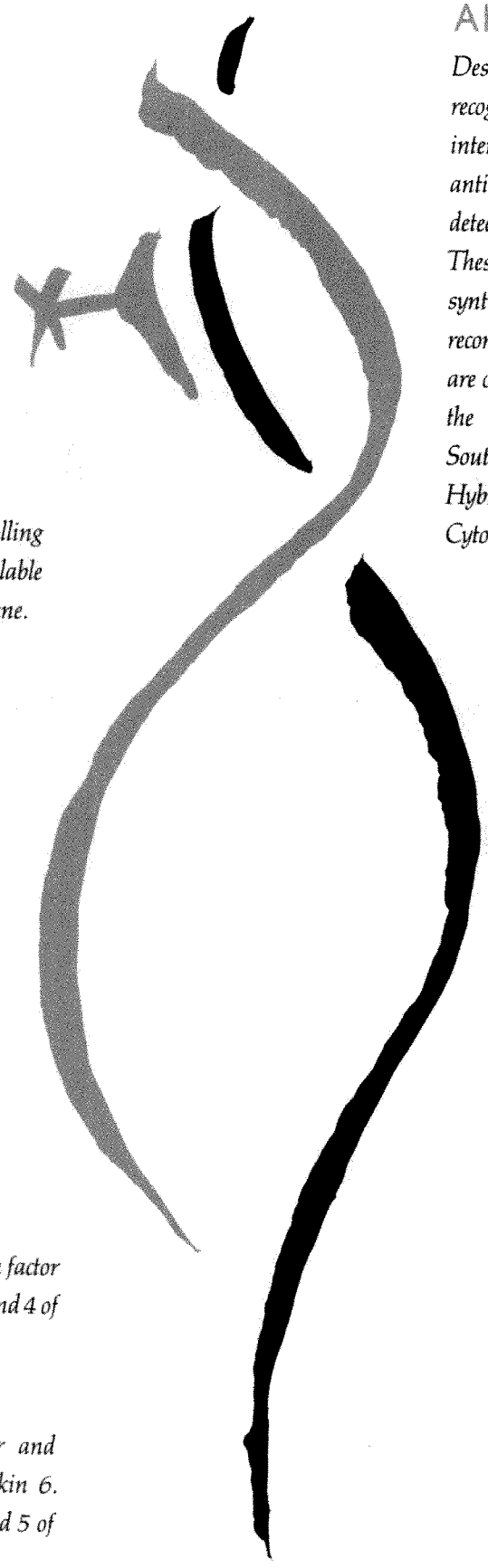
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Answers.

European VLBI in limbo?

The failure of an important and worthy application for research funds to the European Commission demonstrates that Europe needs a more imaginative way of backing research that matters.

SOMETIMES, if rarely, there are research proposals that make perfect sense: the project is sound, indeed imaginative; the applicants have splendid track records, and are prepared to commit their own resources to the project; and there is every reason why the intended source of funds should welcome the proposal both as a vehicle for spending money wisely and because it furthers that organization's wider objectives. What executive officer of a grant-making organization could fail to jump at such a prize? Yet that is what the European Commission has just done.

The circumstances, which are not particularly complicated, concern the development of Very-Long-Baseline Interferometry (VLBI) in Europe. Everybody knows how valuable this technique has been shown to be in the past decade and a half. The principle is simply that of simultaneously pointing two or more radiotelescopes at the same distant radio source. The coordinated instruments then function as one instrument whose resolving power is proportional to the distances by which the instruments are separated (or, strictly, to the ratio of the baseline and the wavelength at which observations are made).

The principle is no different from that in which neighbouring radiotelescopes are deployed in concert to form a radiointerferometer, but there is a practical difference: because of the distances between the instruments, signals from different receivers cannot be compared in real time, but must instead be recorded on magnetic tape and correlated with each other later, using a device known as a correlator.

By now, the achievements of VLBI are widely appreciated. In the observation of distant radio galaxies, the technique promises the most direct observational evidence of what happens near the galaxy-sized nuclei of these often vastly extended objects, thus helping to decide whether the central galaxies are driven by black holes or by some other mechanism. Last year, an observation of the active nucleus of the radio galaxy 3C84 reported an accuracy of 100 microarcseconds (Bartel, N. *et al. Nature* **334**, 131; 1988), no doubt still a record. But there are many more familiar astrophysical processes, such as the mapping of star-forming clouds of gas and dust within our galaxy and the study of the movement of matter near peculiar binary stars, which have been made intelligible by the use of VLBI.

All this was expected at the outset. The surprise has been the usefulness of VLBI in geodesy and geophysics. Coordinated radiotelescopes can so accurately measure the orientation of distant radio sources relative to the Earth's surface that VLBI measurements have become standard measures of the motion of the Earth's poles (including the Chandler Wobble) and of variations in the length of the day.

The point has also been reached at which VLBI is yielding, in what might be called real time, a log of the relative movement of the Earth's tectonic plates. The uncertainty in baseline measurements, inferred from the observation of known distant objects, is already no more than 3 cm, comparable with the annual separation of the plates on either side of the Mid-Atlantic Ridge, and should be further reduced as more high-frequency instruments join the VLBI collaborations and as data analysis is improved. Already pairs of movable radiotelescopes have provided a plausible account of the internal distortion of the tectonic plate of the south-western United States.

But these developments are only a beginning. Coordinated observations by telescopes separated by the diameter of the Earth are commonplace, but now there are ambitions — even plans — to surmount the limitation of the Earth's finite size. The European and US space agencies have made a joint study of an orbiting radiotelescope, while there is a possibility that the Soviet Union may launch such a device in the early 1990s, as may Japan. VLBI, it seems, is booming.

That, sadly, is where recent European disappointments are ironical. From the outset, European radio-observatories have played an important part in wider international collaborations. Most of them now belong to a club, called the European VLBI consortium, which organises collaborations and worries about data acquisition and analysis.

There is the rub. For at least the past three years, it has been clear that existing facilities would soon be overwhelmed by data. Technically, there are two problems — that of recording data accurately in the expected volume (determined by the need to record with high time-resolution and over a range of frequencies) and that of comparing data from different stations so as to extract meaningful information.

This is what prompted the consortium last year to apply to the European Commission for funds with which to equip itself with suitable facilities. The hope had been that a correlator centre could be founded at the Westerbork observatory in the Netherlands, and that the Commission might also help equip individual stations with suitable recording equipment.

As might be expected, the Commission seems to have warmly applauded the proposal, as neat a marriage as there could be of scientific merit with the ideal of European collaboration. Much in the spirit in which censors about to ban books first read them through a second time, the Commission cannot be faulted for the attention which it has given the proposal. But the plan falls outside the terms of reference of the most relevant of the Community's research programmes, the Framework stimulation programme, which, administered by the CODEST committee, exists to bring European researchers together in research collaborations.

In grant-makers' terms, there are several pitfalls buried in the VLBI correlator project, not the least of which is that CODEST's own funds are repeatedly being overtaken by growing demands. But the VLBI correlator would also be a permanent institution, with annual running costs, of a kind that the Commission is not at present equipped (or even empowered) to shoulder. So everybody has been sympathetic, and CODEST has offered the consortium a grant of 300,000 ECU (which it should accept) to improve on its proposal, or even to commission the design of a data-recorder.

This is a sad state of affairs for which nobody is to blame — as yet. While member states delight in playing cat-and-mouse with the Commission over its science budget, it is natural that the Commission should shy from building institutions with implicit promises to support them. It is similarly natural that members of the consortium should obtain from national sources the equipment needed to remain in membership. And nobody can complain that the Commission should seek to make its funds go as far as possible by concentrating on "pump-priming seed-corn projects", to mix two grant-makers' metaphors.

But when, as on this occasion, it finds itself having to turn down a project of outstanding merit simply because its own rules are inappropriately designed, it should do the next most sensible thing — and change the rules.

John Maddox

NMR spectroscopy

Beyond the magic angle

J.M. Thomas

A RECENT technical achievement[1], which entails rapidly spinning a sample in a magnetic field around two axes at the same time, like a top within a top, promises to revolutionize the structural elucidation of solids by nuclear magnetic resonance. Double-rotation NMR is the term coined by the originators of this significant development, A. Pines (Lawrence–Berkeley Laboratory), E. Lippmaa and A. Samoson (Institute of Chemical Physics and Biophysics, Tallinn, Estonia). It should greatly extend the range of magnetic-resonance spectroscopy because it improves, by about two orders of magnitude, the accuracy with which the siting of certain types of atoms and ions in a solid may be distinguished.

NMR had humble beginnings, at least so far as those interested in the determination of molecular structure were concerned. Shortly after its invention in the mid-1940s, it seemed that NMR would be of premier concern to those whose ambitions were accurately to measure the fundamental properties of atomic nuclei. Then came the realization, greeted with growing excitement by chemists, that the precise frequency with which a given nucleus (of finite spin) resonates in an applied magnetic field is a delicate function of the local electronic environment of that nucleus. This gave birth to the notion of chemical shift. For example, protons (which are spherical, spin 1/2), resonate at frequencies governed by the chemical groupings to which they are attached: the resonating frequencies of –HCH–, –CH=CH$_2$, –C≡CH and –CO–OH are very different from one another. For other spherical nuclei the range of chemical shifts is large; and ^{13}C NMR readily distinguishes between aliphatic and aromatic carbons, just as ^{29}Si spectra discriminate between the 4- and 6-coordinated element. This is one of the main reasons why NMR is an indispensible structure-determining tool in the physical and biological sciences.

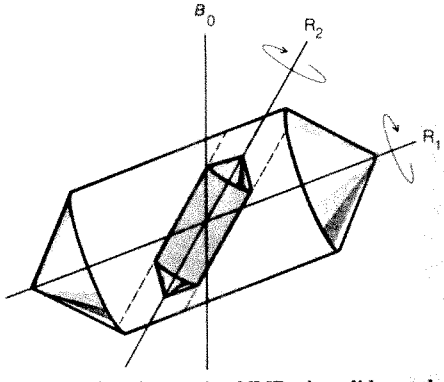


Fig. 1 In double-rotation NMR, the solid sample is rotated at an angle to the axis of the holder which is itself spun at an angle, the magic angle (54.74°), to the applied field B_0. The angle between R_1 and R_2 is 30.56°. Together these rotations remove dipolar and quadrupolar line broadening. (From ref. 1.)

In NMR spectroscopy one measures, *inter alia*, the radio frequencies emitted by, and the rates of realignment of, nuclear spins in a magnetic field after they absorb energy from radio-wave pulses. Because of random motion executed by entities in the fluid state, anisotropic magnetic and electrical interactions are averaged out so that high-resolution spectra may be routinely obtained with liquids and solutions. With solid samples there is no such averaging and the NMR spectra are hugely broadened. For a long time the prospects of retrieving chemical shift information from solid-state NMR spectra looked bleak until, in 1958, Andrew *et al.*[2], at the University College of North Wales, and Lowe[3] realized that the blurring effects of spectral resonances are represented mathematically by a term that contains the factor $(1-3\cos^2\theta)$. By spinning the sample about an axis at an angle $\theta = 54.74°$ (equals $\cos^{-1} 1/\sqrt{3}$) to the magnetic field, the solid behaved as if it were an isotropic liquid, and high-resolution spectra of a quality comparable to those achievable with liquids could be obtained.

Although magic-angle-spinning (MAS) NMR has been of enormous value in recent years in the study of solids[4,5] in general, and of absorbents and catalysts[6] in particular, as well as of a wide range of quasicrystalline and membranous materials — for which competing techniques such as X-ray crystallography are not as well suited — it has hitherto suffered from one rather serious limitation: quadrupolar nuclei (non-spherical ones, with spin greater than 1/2, like ^{11}B, ^{17}O, ^{23}Na and ^{27}Al), which are more numerous than spin-1/2 nuclei, give rise to broadening influences that are not eliminated by spinning at the magic angle of 54.74° nor by resorting to other strategies such as radio frequency manipulation of the spins.

This has meant that many problems of great interest to the solid-state chemist and physicist and to the materials and earth scientist have remained beyond the reach of high-resolution solid-state NMR. One practical, though costly, way of minimizing this limitation is to employ ever-higher magnetic fields, as second-order quadrupolar broadening effects are inversely proportional to the field strength. This approach has indeed been successful[7] in the study of zeolites, for which ^{27}Al MAS NMR spectra recorded at 11.744 tesla, but not at lower (7.946 or 9.359 tesla) fields, resolve two crystallographically distinct tetrahedral framework sites.

But double-rotation NMR offers an alternative route forward. By employing two 'magic angles' — the customary one of 54.74° and a new one of 30.56°, appropriate for the quadrupolar nuclei — essentially all the blurring effects that broaden the resonances are eliminated as a result of the complex motion to which the sample is subjected (Figs 1 and 2). The powdered solid rotates inside a holder which simultaneously rotates on a second axis, air jets and air bearings being used to achieve rotation speeds of up to 6 kilohertz.

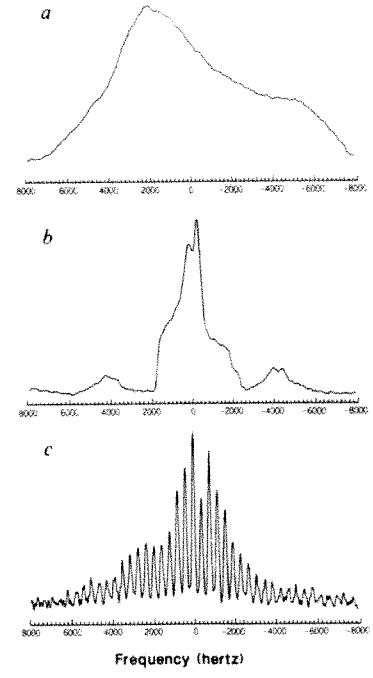


Fig. 2 The NMR spectrum of ^{23}Na in polycrystalline sodium oxalate, from a static sample (*a*), a magic-angle-spinning experiment (*b*) and a double-rotation experiment (*c*). The linewidth is reduced from 12,000 to 4,000 to 140 hertz, respectively, as dipolar and quadrupolar broadening are suppressed. The sidebands in *b* and apparent 'structure' in *c* are related to the spinning frequencies. (From ref. 1.)

The Berkeley–Tallinn team has already demonstrated that the technique works for ^{23}Na nuclei, two well-resolved signals (of width 75 hertz) being obtained from a mixture of the sulphate and oxalate of sodium. With the very recent report (A. Pines *et al.*, preprint) that the mineral diopside (isotopically enriched) yields three distinct ^{17}O signals, the stage is now set for exciting developments in the elucidation of structures and mechanisms of interest to the earth scientists, materials engineers and those investigating catalysts, adsorbents and warm superconductors. There is a paucity of techniques capable of probing directly the precise electronic nature of oxygen ions in such solids

Electron-energy-loss spectroscopy (oxygen K-edge structure), X-ray spectroscopy and electron spin resonance are rather specialized though quite useful tools. But ^{17}O double-rotation NMR is potentially more powerful.

For zeolite-rho, faujasite (zeolites X and Y) and ferrierite, all of which are important solid catalysts, there are known to be, respectively, two, four and eight crystallographically distinct oxygen sites. Will these be resolved by double-rotation NMR (like the 24 silicon sites[8] in the ^{29}Si MAS NMR spectrum of ZSM-5)? And will there be only one peak in the ^{17}O double-rotation NMR spectrum of sodalite? Or more, if there are Al–O–Al linkages[9] present? These answers will determine the new directions which solid-state NMR will give to the structural elucidation of new zeolitic and related catalysts. Likewise, with ^{17}O enriched superconducting ceramics, apart from identifying crystallographically distinct oxygens, one may hope to find direct evidence for the so-called charge-disproportionation mechanism ($O^{2-} + O^0 \rightarrow 2O^-$) or the existence of the previously postulated peroxy, ionic species, O_2^{2-}. These issues need to be clarified if we are to understand the transport mechanisms in the various families of oxide superconductors. □

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Telomerase

The beginning of the ends

Jack W. Szostak

EVERY time a linear chromosome is replicated it is in danger of being whittled away from both ends. The origin of this danger lies in the requirement of DNA polymerases not only for a template to copy but also a primer to extend: because polynucleotide synthesis can proceed in only one direction, from 5′ to 3′, this leads to a problem in copying the 3′ ends of both strands of a linear chromosome, where there can be no primer to extend (see figure). A common solution to this problem is to have simple repeat sequences at the telomeres (ends) of linear chromosomes, and a specialized system for generating additional repeats, compensating for loss in replication. Greider and Blackburn isolated and characterized[1,2] an enzyme that can synthesize telomeric repeats; notably, this telomere terminal transferase has an essential RNA component. The same authors, on page 331 of this issue[3], find that the RNA component of the telomere terminal transferase of *Tetrahymena* contains the complementary sequence of the telomeric repeats that it synthesizes, strongly suggesting that the enzyme is a remarkable kind of DNA polymerase that carries its own template.

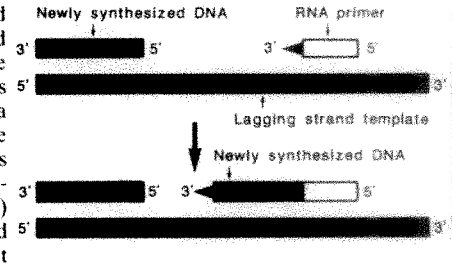
The existence of telomerase was originally hypothesized[4] to explain several bizarre properties of telomeres. Synthesis of new telomeric repeats has been observed in three very different organisms. In *Tetrahymena* and other ciliates, chromosomes are fragmented and new telomeres are formed spontaneously during the formation of macronuclei. In yeast, linear plasmids made *in vitro* with *Tetrahymena* telomeres can be elongated *in vivo* with newly synthesized yeast telomeres. And in trypanosomes, telomeres elongate steadily at about one repeat per generation during somatic growth. Of course, telomerase also provides a ready explanation for the maintenance of normal telomere length in the face of incomplete replication.

The *Tetrahymena* telomerase is a ribonucleoprotein that adds GGGGTT repeats to short, single-stranded, synthetic oligonucleotides. Only telomere-like sequences are elongated; random-sequence oligonucleotides are ignored by the enzyme. How is this specificity obtained? Greider and Blackburn[1–3] cloned and sequenced the RNA component of telomerase, and find embedded within it the sequence CAACCCCAA — one and a half repeats of the complement to the synthesized sequence. Furthermore, they show that the enzyme can be inhibited by oligonucleotides complementary to this sequence, and inactivated by RNaseH cleavage at this site. Even more remarkably, an oligonucleotide that lines up just 5′ to this sequence, but contains no telomeric repeats itself, can be elongated by the enzyme, further implicating the RNA as a template[3]. No doubt we shall soon see the sequences of corresponding RNAs from related organisms with different telomeric repeats; if the RNA does indeed act as a template, each one should contain the appropriate complementary sequences. An even more decisive experiment would be possible if such an RNA template could be found in yeast: replacement of the resident gene with one modified to change the template sequence should generate yeast with any desired telomere sequence.

But do all eukaryotes replicate their telomeres with the aid of telomere terminal transferase? Similar enzymes have been detected in other ciliates — *Oxytricha*[5] and *Euplotes* (Shipper-Lentz, D. & Blackburn, E.H., submitted) — so the role of this enzyme in these organisms seems assured. But it has not yet been detected in yeast, despite several attempts to find it. This is not necessarily surprising — the ratio of telomeres to total genomic DNA is about 1,000-fold less in yeast than in ciliates, so there may simply be very little of the enzyme in yeast. Still, most eukaryotes never need to generate telomeres *de novo*, and might perhaps be able to use some sort of recombinational process to elongate them. In such models, a telomeric 3′ end could invade at an internal site in another telomere, where it could be elongated by polymerase on the existing template. Such a gene conversion-like process could lead to the same elongation as is achieved by telomere terminal transferase. Indeed, recombination is almost certainly responsible for maintaining the average telomere length of *Tetrahymena* mitochondrial DNA[6].

Does recombination actually play a role in telomere elongation in yeast? Pluta and Zakian[7] have done a cleverly designed experiment in which they prepared hybrid linear DNA molecules with CCCCAAAA repeats at one end and CCCCAA repeats at the other. After transformation into yeast, each end is elongated with yeast $C_{1-3}A$ sequences, as expected. Hybridization analysis of the resulting linear plasmids reveals that in many cases CCCCAAAA sequences have been transferred to the CCCCAA end, and vice versa.

How has this happened? Pluta and Zakian suggest that recombination between the ends has taken place and,



Eukaryotic DNA replication. Each new strand of DNA is synthesized on a template provided by one of the old strands (only one strand, the lagging strand, is shown here). But synthesis can be initiated only in the presence of a primer, which is provided by a 10-nucleotide RNA primer synthesized about 200 nucleotides 3′ to the replication fork. DNA is thus synthesized in short fragments (Okazaki fragments) from 5′ to 3′, the RNA primers being degraded and replaced with DNA as each fragment reaches the one preceding it. The 3′ ends of the DNA strands cannot be replicated in this way, however, because there is no more DNA left on which to synthesize the RNA primer.

furthermore, that similar recombination events involving yeast telomeres could be responsible for the subsequent addition of the $C_{1-3}A$ repeats, and for telomere elongation in general. Because their observed events are independent of the *rad 52* gene product, which is required for all other known recombination events involving double-stranded ends, some new, perhaps telomere-specific, system could be involved, if indeed recombination is responsible for these events.

Work from my laboratory argues against an essential role for recombination in telomere elongation[8]. We were surprised to find that 20–60 base pairs of non-telomere DNA can commonly be retained in between CCCCAA repeats present on the input DNA and the $C_{1-3}A$ DNA added on in yeast. Because these linker sequences are unrelated to telomeric repeats, the subsequent addition of $C_{1-3}A$ repeats cannot involve homologous recombination.

Our results also provide an alternative explanation for the results of Pluta and Zakian[7]. A resolution reaction that can separate ligated telomeres is well-documented in yeast[9]. If the two different telomeres became ligated in yeast, and then resolved by breakage within one of the stretches of telomeric repeats, the observed movement of DNA would take place. Pluta and Zakian reject this explanation because the transferred segment would be inverted relative to the normal telomeric orientation. Telomeric sequences in the wrong orientation are known not to be elongated in yeast. In the light of our results[8], however, it is likely that a short stretch of inverted telomere DNA would not block subsequent elongation. The only way to resolve this point is to clone and sequence several examples of the chimaeric telomeres to determine the orientation of the transferred segment. □

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Molecular evolution

What, if anything, is *Prochloron*?

David Penny

IT is almost 20 years since the suggestion[1] that free-living photosynthetic prokaryotic organisms containing chlorophylls *a* and *b*, but lacking phycobilin pigments, existed a billion years ago. These hypothetical organisms would be ancestors of the chloroplasts of green plants. At that time, the notion seemed reasonable, but scarcely testable. I still remember the excitement five years later when a possible member of such a group was found alive and well, living in Baja California, Mexico[2]. The organism was named *Prochloron*, and the group prochlorophytes. But the first work[3] comparing fragments of nucleotide sequences from ribosomal RNA of these organisms did not support prochlorophytes being the ancestor of green chloroplasts. Rather, it seemed that chlorophyll *b* had developed separately in *Prochloron* and chloroplasts. Progress in clarifying the position of *Prochloron* has been slow because it cannot be grown in culture. Recently, a free-living form *Prochlorothrix*, a presumed relative of *Prochloron*, has been discovered and cultured[4]. On pages 380 and 382 of this issue[5,6], two groups report on the evolutionary position of *Prochlorothrix* but reach apparently conflicting conclusions.

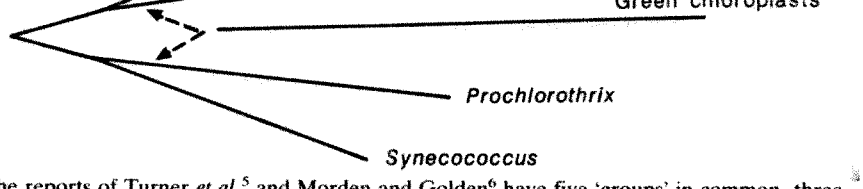
Turner *et al.*[5] compare the nucleotide sequences of the small-subunit (16S) RNA from *Prochlorothrix*, chloroplasts and cyanobacteria. They use distance methods for building evolutionary trees and estimate the accuracy of their results by 'bootstrapping'. Their trees clearly separate green chloroplasts from *Prochlorothrix*, in agreement with the earlier results[3]. In this scheme, the closest relative of a chloroplast ancestor would be the 'chloroplast' (cyanelle) of the protist *Cyanophora*, which has typical cyanobacterial structure and biochemistry. The interpretation is that the loss of phycobilins, and the gain of chlorophyll *b* has occurred at least twice.

Morden and Golden[6], on the other hand, use DNA sequences from a photosynthetic membrane protein (*psbA*) from photosystem II. They use the position of deletions in the molecule as well as trees built using the parsimony (minimum mutations) criterion and come to the opposite conclusion, namely that the prochlorophytes such as *Prochlorothrix* are the ancestors of the green chloroplasts. Similarities and differences between the reports[5,6] are shown in the figure.

Such an impasse demands comment. First, neither group supports the idea[7] that prochlorophytes are a very ancient group that evolved at the same time as cyanobacteria. It is small consolation that at least the two sequences put *Prochlorothrix* into the same main taxonomic group. Putting aside for the present special pleading such as lateral transfer, can we predict which conclusion will eventually be proved correct? Unfortunately, no.

Two strengths of the method of Turner *et al.*[5] are bootstrapping to give an estimate of errors and a correction for unequal rates of change on different nucleotides. But the method could be sensitive to unequal rates on different lineages[8]. Turner *et al.* have two sets of biochemical data, RNA sequence and photosynthetic pigments (including information on biosynthetic pathways and chlorophyll-binding proteins). If the two sets of data do agree then it is valid to say they support the same tree. If they do not then it is difficult to justify ignoring completely one or the other. The authors appear to assume their RNA tree is correct and give a zero weighting to the pigment data.

Another possible difficulty is that there could be some convergence of function in 16S RNA sequences in chloroplasts. This could lead to the *Cyanophora* and chloroplast sequences converging sufficiently to come together on the tree. An example of functional convergence has been found with lysozyme in two groups of mammals



The reports of Turner *et al.*[5] and Morden and Golden[6] have five 'groups' in common, three standard cyanobacteria, *Prochlorothrix* and chloroplasts from green plants. Assuming the differences in isolates are unimportant, the first four give the same rooted tree for 16S RNA and the *psbA* gene. Thus the difference is not in the position of *Prochlorothrix* but rather in the green chloroplasts. The 16S-RNA results[5] derive the chloroplasts from near *Synecococcus*, which requires chlorophyll *b* to have arisen twice as a photosynthetic pigment. The *psbA* results[6] derive chloroplasts from 'prochlorophytes', thus giving a single origin of chlorophyll *b*.

with ruminant digestion, ungulates (including cattle) and Langur monkeys[9]. If convergence has occurred, then one test is to run the RNA data without *Cyanophora*. *Prochlorothrix* should now join with chloroplasts. But there is other evidence[10], observations of the ultrastructure, for example, that could relate the *Cyanophora* group to the green plants and support the tree of Turner *et al.*. It would also be interesting to determine the sequence of some anomalous protists that have chlorophyll *b*, such as *Chlorarachnion*[11].

Morden and Golden[6] find that the two types of biochemical data do agree, but apart from omitting nucleotides in the region of the deletion, they do not test the accuracy of their tree. Parsimony methods are susceptible to error from long edges coming together[8]. Sequences from chloroplasts of the ancient green algal group Prasinophyceae may be particularly helpful in resolving this question. In addition, Morden and Golden[6] report a smaller selection of cyanobacterial sequences (7 sequences from 5 taxa compared with 17 RNA sequences). It is still possible that additional *psbA* sequences from cyanobacteria would fall between green chloroplasts and *Prochlorothrix* and thus separate these two groups. It is therefore important to determine more sequences, including the *Cyanophora* cyanelle.

How can the impasse be resolved? I have made suggestions for additional sequences (or temporarily deleting sequences). It is always safer to reconstruct trees by more than one method, wherever possible using more than a single molecule. One method with one coding sequence seems insufficient to reconstruct unambiguously the whole of evolution. Methods such as convergence, bootstrapping and jackknifing[12,13] are available to help estimate accuracy.

Where, then, do we stand? The biggest difficulty is in persuading people that collecting sequences is the easy part of any study. The hard part is estimating the accuracy of the results. In some areas of molecular biology it is accepted that, for example, establishing secondary and tertiary structure from sequences is much harder than obtaining the primary sequences. Any measurement, to be scientific, must include an indication of its accuracy. Despite the progress that has been made, reconstruction of evolutionary trees from sequence data must still appear to those in other fields as parascientific. □

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Evolutionary ecology

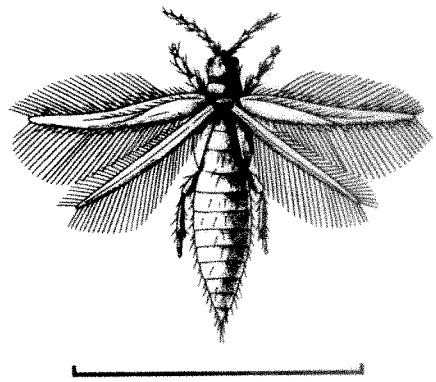
Diversity of little things

Jon Seger

We are gigantic animals. If there are 10 million species (probably a conservative estimate[1,2]), then we are among the largest 0.001 per cent. Songbirds, mice and honeybees seem small to us, but they are almost certainly in the top 1 per cent. The remaining 99 per cent consists mainly of insects and mites with average body lengths of roughly 3 mm[2,3] (the size at which we cease to notice things even when they land in our soup). An immense diversity of life-histories and reproductive biologies would be expected to occur at these sub-sub-lilliputian scales, simply because there are so many species. In fact, the action appears to be disproportionately concentrated among very small animals. To cite just one of many possible examples, parthenogenesis in its many varieties is overwhelmingly most common among small arthropods[4,5]. The report by Crespi on page 357 of this issue[6] describes what is apparently the first known instance of facultative viviparity, in which individual females can lay eggs, or give birth to live young, or both. This feat is performed by a 5-mm thrips.

Thrips (see figure) belong to the insect order Thysanoptera ('fringed wings'). Most species are small to very small (5–0.5 mm), and most live on plants. The order is thought to be primitively haplodiploid, in the style of Hymenoptera, with haploid males arising from unfertilized eggs (arrhenotoky) and diploid females arising from fertilized eggs. Completely asexual reproduction is known in some species, but most seem to be fully sexual (on the female side). Most species lay eggs, but some are wholly or partially viviparous.

In the species studied by Crespi (*Elaphrothrips tuberculatus*), reproductive mode is associated with sex of offspring: male offspring are produced viviparously and female offspring oviparously. During a given bout of reproduction, a female is



A typical thrips from the order Thysanoptera. Scale bar, 1 mm.

either viviparous or oviparous. But by placing females in fine-mesh bags, under otherwise natural conditions, Crespi demonstrates that many long-lived females switch modes, thereby producing offspring of both sexes.

Because sex of offspring is associated with reproductive mode, and the sex ratio is subject to powerful frequency-dependent selection, roughly equal numbers of females should be found in each mode at any given time. Crespi's study population has two generations per year. In the summer generation there is a slight excess of male-producing, viviparous females, but in the spring there is a large (3:1) excess of female-producing, oviparous females[6–8]. If this pattern of sex allocation represents an evolutionary equilibrium, then the population probably experiences local mate competition[9] (differential migration of the sexes) or partial bivoltinism[10,11] (differential survival of the sexes between generations), or both.

Adult males guard gravid oviparous females for hours or days at a time, but they usually ignore viviparous females[7]. This behaviour is consistent with the assumption that *E. tuberculatus* is haplodiploid, because male offspring have no fathers under haplodiploidy.

Viviparous females have only about half the fecundity of oviparous females, but newly laid eggs suffer considerable mortality before hatching. In the end, each reproductive mode yields roughly the same number of pupal offspring. Why, then, should male offspring be produced viviparously? One obvious adaptive hypothesis is that males benefit more than do females from entering the world in an advanced state of development. Crespi has previously shown[7] that because success at mate-guarding is strongly size-dependent, males benefit more from being relatively large than do females.

But if being produced viviparously is good for males, surely it should be good for females as well. Why, then, are female offspring not produced in the same way? A plausible nonadaptive hypothesis is simply that the evolutionary transition to viviparity is easier under parthenogenesis than it is under sexual reproduction,

perhaps because the egg must be fully ovulated before it can be fertilized. Within the arthropods as a whole there seems to be a strong association between viviparity and parthenogenesis (N.A. Moran, personal communication); this pattern is particularly striking in cyclical parthenogens such as aphids and *Daphnia*. Even so, some taxa manage to combine viviparity and sexual reproduction (for example, parasitoid flies that 'larviposit' on their hosts). It will be interesting to see what turns up in thrips as more species are studied in detail.

Large animals easily excite our emotions; small ones probably would if we knew them better. They make up most of the Earth's biotic diversity, in several senses of the word, and they remain largely unnamed and unstudied. This means that many thousands, even millions, of small species may be driven to extinction through habitat destruction[12] before we even note their existence. But millions more are likely to remain, still the richest part of "life on a little-known planet"[13]. □

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Oxide superconductors

Electron doping tests theories

V. J. Emery

THE discovery of electron superconductivity in cuprate compounds, reported by Tokura *et al.* on page 345 of this issue[1] has significant implications for our understanding of high-temperature superconductors. The charge carriers in previously known cuprate superconductors can be thought of as holes, or missing electrons. Typically an insulating material is rendered metallic by substituting divalent ions for trivalent ones (say, Sr^{2+} for La^{3+}) or by adding oxygen, which enters the compound as O^{2-}: to maintain charge balance, electrons are removed from the (superconducting) copper oxide planes. Tokura *et al.* went in the opposite direction. Starting from Ln_2CuO_4, where Ln is one of the lanthanides Pr, Nd, or Sm, they replaced Ln^{3+} by Ce^{4+} and removed oxygen by annealing under reducing conditions. In this way electrons are added to the CuO_2 planes, and superconductivity is achieved when the carrier concentration exceeds about 20 per cent per formula unit.

At this early stage, rather little is known about the electronic and magnetic properties of the new materials produced by Tokura *et al.*, although they surely will be studied closely during the next few months. But because the supercurrent is carried by electrons rather than holes and because of the planar copper coordination (see below), the new materials allow new kinds of tests of theories of high temperature superconductivity.

The simplest possibility is that the CuO_2 planes in the insulating parent compounds, Ln_2CuO_4 and La_2CuO_4 turn out to be particle–hole symmetric, that is they behave in the same way if electrons are added or removed. Indeed this is a property of 'single-band' models[2–4] of the CuO_2 planes, which neglect all the electron states in the material (or energy bands) apart from a partially occupied band bearing the delocalized charge carriers. In some ways this would be the least interesting, but nonetheless important, outcome, replicating properties already observed in existing materials.

An alternative point of view[5] is that the Coulomb interactions between the charge carriers are so strong that other bands cannot be ignored. In the parent compounds the O($2p$) levels are filled and the holes are in Cu($3d$) states, giving Cu^{2+} ions with a spin that is responsible for the observed magnetic behaviour. When holes are added by doping they go into ($2p$) orbitals because the strong Coulomb repulsion makes Cu^{3+} extremely unfavourable energetically. Because the O($2p$) orbitals become partially filled, the added holes can form a conduction band which ultimately leads to superconductivity. Of course the situation is not so extreme, there is some hybridization (mixing of states), but to talk of localized moments on copper and mobile holes on oxygen is a convenient 'zeroth order' way of speaking.

There is much evidence[6,7] in favour of this picture mainly from X-ray, electron and ultraviolet spectroscopy as well as from neutron-scattering experiments. Indeed, the outstanding issue is not so much whether the holes are on oxygen but rather which of the possible $2p$ states are populated. Electron doping would give a different result, however, because the O($2p$) level is filled in the parent compound, and the electrons must go onto copper sites to produce Cu^+. This conclusion could be verified by X-ray absorption near-edge spectroscopy[6] for example. It is clear that the T' structure of the new materials[1] (in which each copper ion sits in a square plane of oxygen ions) is electrostatically favourable for electron doping onto copper: unlike other structures there are no apical O^{2-} ions sitting directly above or below the copper sites repelling the excess charge.

Naively, the closed-shell Cu^+ ions behave like vacancies (missing spins) in the array of local Cu^{2+} moments which are magnetically coupled with a strength J. The vacancies are mobile because an electron can hop with amplitude t from Cu^+ onto a neighbouring Cu^{2+}. This is formally identical to the so-called t–J model[8] which may be derived from the single-band model[2–4] in the limit of strong interactions, and has been widely used to study magnetic mechanisms of superconductivity. In this way, the simplest versions of the two models might coincide for electron doping. At the same time, there is an ongoing debate about whether, for some purposes, the same may be true for hole doping. Specifically, for low-energy properties, such as magnetism, the mechanism of superconductivity and parameters of the superconducting state, does a hole on oxygen behave in the same way as a missing spin[9,10]? If so, there would be a limited and more elaborate form of particle–hole symmetry. The new materials may shed some experimental light on this important question.

A study of magnetic properties will be particularly interesting in this regard. It is already known from neutron-scattering experiments by Torardi *et al.* (D.E. Cox, personal communication) on Pr_2CuO_4 and muon-spin-rotation ones by Luke *et al.* (Y. Uemura, personal communication) on Pr_2CuO_4, Nd_2CuO_4 and Sm_2CuO_4 that the end members have magnetic order. For Pr_2CuO_4, the ordering temperature is about 260 K and the magnetic structure is similar to that of La_2CuO_4, with an ordered moment of about 0.5 Bohr magnetons per copper atom (Cox). An extension of these studies to the doped materials will enable us to contrast the effects of holes on oxygen and electrons on copper

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as the magnetic order gives way to superconductivity.

Of course this naive description of electron doping is not the whole story. A Cu^+ ion will polarize its environment, giving rise to some density of holes on the surrounding oxygen sites. Just as for hole doping, the charge carriers are complex and extended entities and there are more routes to superconductivity than the simple *t–J* model allows. Many proposed mechanisms will require rethinking. Those relying on a displacement of the position or the charge of apical oxygens or the associated splitting of the Cu(3*d*) levels are clearly ruled out. Others emphasizing charge transfer between copper and oxygen must adapt to the new location of the charge carriers. □

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Astronomy

Mass loss from Wolf–Rayet stars

Roger J. Tayler

WOLF–RAYET stars are very massive stars which are undergoing very rapid mass loss, typically several times 10^{-5} solar masses ($M_\odot$) per year[1]. Although most investigators believe that the mass loss is primarily driven by the pressure of the star's radiation, the momentum being communicated to the flow by very strong absorption in selected spectral lines, there is not complete agreement on this point. In a recent paper, Moffat *et al.*[2] report observations of five Wolf–Rayet stars in which the mass loss has an irregular blob-like structure and they believe that this property might help to distinguish between various proposed mass-loss mechanisms.

It has become clear during the past 20 years that a very wide variety of stars experience mass loss at rates ranging from the solar wind of a few times $10^{-14}\ M_\odot\ yr^{-1}$, or less, to the dramatic loss of many $M_\odot$ in the explosion of a supernova. In most cases the mass loss becomes apparent through the existence of very broad emission spectral lines which can be understood only in terms of mass leaving the star; in some cases direct observations are ambiguous as regards infall or outflow but there are usually other features which resolve the ambiguity. The most simple theoretical discussion of mass loss assumes that it is steady and spherically symmetric and such a discussion gives a first qualitative understanding of what is happening. It is, however, by no means certain that mass loss has this property.

For example, if the star is rapidly rotating or possesses a significant magnetic field, it will not be spherical and it is therefore unlikely that any mass loss will have spherical symmetry. If the mass loss is initiated by pulsation of the star, there will be time variations. Finally, a spherically symmetrical outflow might be thermally unstable, leading to the formation of cool dense blobs in a hotter diffuse flow. Evidence for such departures from the simple model can be found by studying the detailed profiles of the spectral lines which provide evidence of mass loss.

In the case of stars with low surface temperatures such as the Sun, the mass loss is strongly influenced by, if not determined by, the structure of the magnetic field at and above the star's surface. The field is maintained by a dynamo process in the star's outer convection zone and its strength seems to depend on the rotation speed of the star. The field structure is complex with some magnetic field lines forming closed magnetic loops within a few stellar radii and with others extending to large distances from the star. It is not surprising that in this case the mass loss is not spherically symmetrical. It is concentrated along the open field lines, while the energy input at the base of the closed loops raises the confined gas to much higher temperatures. Interestingly, in the case of one cool star, AB Doradus, Robinson and Collier-Cameron[3] have shown that the gas confined by the loops has apparently suffered thermal instability so that cool clumps of matter are embedded in the hot gas.

Moffat *et al.*[2] now show, by a study of emission lines of helium in five hot Wolf–Rayet stars, that their mass outflow must contain blobs of material. What they have observed are bumps in the spectral line profiles, which have the property that those starting redshifted relative to the line centre become more redshifted with time, while those starting blueshifted become more blueshifted with time. Their conclusion is that they are seeing blobs of material which are being accelerated outward, some on the near side of the star and some on the far side. Their study of the time of appearance of the blobs indicates that they cannot be related to any fully predictable property of the star, such as rotation or magnetic field structure or a regular pulsation. The blobs appear to arise randomly in starting time and in direction outward in the flow and this can probably be understood in terms of a stochastic instability of a radiatively driven wind as was proposed by Rybicki[4].

In his discussion, Rybicki[4] developed work by other authors on various possible instabilities in outflowing winds. The sensitive velocity dependence of radiative driving due to absorption in a spectral line is a particularly important source of instability. An element of material moving faster than neighbouring elements Doppler shifts the spectral line to a wavelength which has not yet suffered absorption and this leads to stronger driving and instability. Because of the outward motion such instabilities are rapidly advected away and they cannot form a coherent pattern around the star. This appears to accord with the observations of Moffat *et al.*[2], although further work will be needed to determine whether quantitative agreement between theory and observation exists. □



A Wolf–Rayet star surrounded by the nebula NGC2359 in the southern skies.

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Agricultural genetics

Moving in on plant genes

Robert Shields

VIRTUALLY nothing is known about the protein products of the genes controlling morphology, dormancy, photoperiodicity and disease resistance in plants. Two techniques being used to try to clone these genes are transposon tagging and gene mapping. Transposons (mobile pieces of DNA) can generate different phenotypes which should allow the genes governing the phenotype to be isolated; and mapping should allow specific genes to be targeted. Although tagging is the more elegant technique, a new report[1] by Young *et al.* indicates that mapping may be the first method by which one of these genes is uncovered.

Transposons can be used as simultaneous mutagens and gene tags. Several plant genes (mainly those involved in pigment biosynthesis) have been cloned in this way from maize or snapdragon. The discovery[2] that the transposon Ac in maize could be transferred to other plants, where it is moved from its original site of insertion to a new locus, was therefore met with considerable excitement. Self-fertilization of the resulting plant will give the tagged locus in a homozygous state and simultaneously reveal a new phenotype. But things may not be that straightforward. In its native host, Ac tends to excise and then reinsert at a closely linked site on the same chromosome[3]. If this is true in the heterologous host, it will be necessary to produce plant lines with transposons inserted close to target genes before tagging can begin. In other organisms such as the fruitfly *Drosophila*, where transposons act as mutagens, specific loci are mutated at vastly different rates as the elements show strong site-specificity for insertion[4]. So, although it may be possible to isolate plant genes by transposon tagging, there may be problems in approaching specific loci by this method.

In several plant species, genes have been assigned to positions in genetic linkage groups which in a few plants can be correlated with cytologically identifiable chromosomes. The resolution of many of these linkage maps is poor and several laboratories are attempting to improve them by constructing maps based on restriction fragment length polymorphisms (RFLPs). These maps have a use as a practical breeding aid, for if an RFLP marker shows close linkage with a desirable gene then progeny in breeding experiments can be rapidly screened for its presence without the necessity for large-scale field trials. A secondary use of such maps is to use them as starting points for attempts to isolate the linked gene.

The first requirement is to position the target gene accurately on the RFLP linkage map and if necessary find new RFLPs which map closer to the gene. Young *et al.* have now elegantly demonstrated[1] the combined use of RFLPs and near-isogenic lines to find markers very close to a plant disease-resistance gene. Plant breeders often cross a cultivated variety with an alien species carrying a desired gene and then backcross to the cultivated variety (the recurrent parent) while selecting to maintain the required trait. After a series of such crosses, the genome of the selected plants will consist almost entirely of the genome of the recurrent parent with a small segment surrounding the target gene which comes from the alien genome. By examining RFLP differences between the original variety and the new near-isogenic line, it is possible to identify RFLP markers lying very close to the introgressed gene. Young *et al.* find markers lying 0.4 ± 0.4 centimorgans (= 2 × 10^5 base pairs in tomato) of the *Tm2a* gene (a dominant gene giving resistance to tobacco mosaic virus by restricting cell-to-cell spread) which had been introgressed into the cultivated tomato (*Lycopersicon esculentum*) from the wild variety *L. peruvianum*. Near-isogenic lines should be extremely valuable for rapidly assigning introgressed genes to their map position.

The correlation between the genetic and the RFLP map is limited by the frequency of allelic variation in the organism examined (which determines the likelihood of finding RFLPs) and the number of cases that can be examined where the target gene and closely linked RFLPs are segregating (which gives the map position). In practical terms 1 centimorgan (1 per cent recombination) is close to the limit of resolution achievable without examining a prohibitively large number of crosses unless near-isogenic lines can be used to give closer linkage. The table shows that for plants such as *Arabidopsis*, which has a very small genome, 1 centimorgan represents 139 kilobases, thus chromosome walking from an RFLP marker to a genetic loci is just within the bounds of possibility, given a lot of effort. Doubtless other genes will be isolated in this way from *Arabidopsis* and will then be used as probes to find homologous sequences in other plants. But it seems unlikely that genes governing many complicated plant functions (such as disease resistance) would be similar between *Arabidopsis* and economically important crop plants.

The resolution gap in plants

Species	Genome size (kilobase pairs)	Map length (centimorgan)	Kilobase pairs equivalent to 1 centimorgan	Ref.
Arabidopsis	7×10^4	501	139	10
Tomato	7.15×10^5	1,400	510	1
Human	3×10^6	2,708	1,108	11
Maize	3×10^6	~1,400	~2,140	12

Map lengths are based on published RFLP data. The conversion of centimorgans to kilobase pairs is an average value as recombination frequency varies in different chromosome regions.

For such plants a 'resolution gap' exists between the genetic and the physical map which must be bridged if their genes are to be approached from linked RFLP probes. A similar (although less daunting; see table) resolution gap in the human genome has spurred technical developments such as pulsed-field gel electrophoresis[5] and cloning in yeast artificial chromosomes to handle and map very large pieces of DNA[6,7] as well as the use of 'jumping' and 'linking' libraries to overcome the necessity of walking everywhere in the genome[8,9]. Once the region surrounding the target gene is physically mapped, ways must be found to identify the gene itself. □

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100 years ago

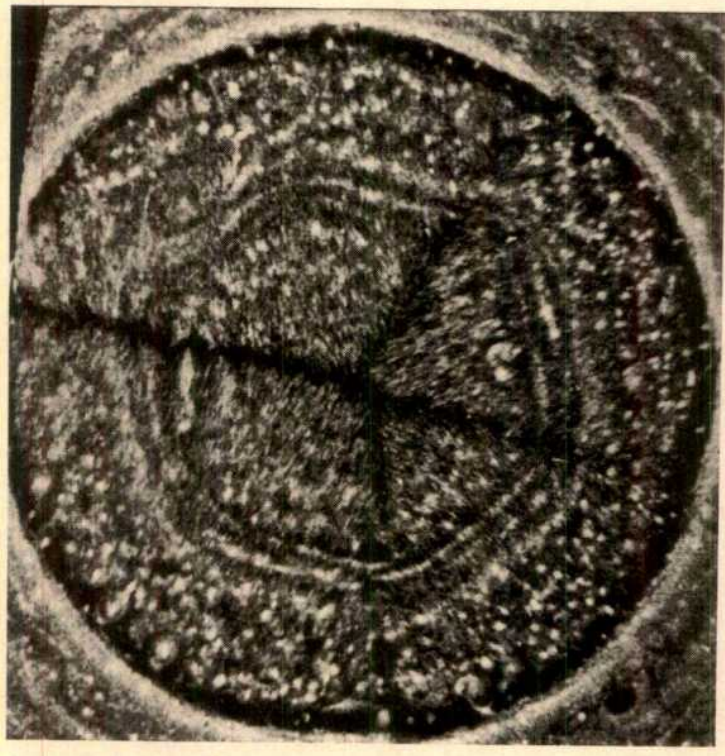
WITH reference to A. C. Haddon's account of the use of the *Remora* or sucker fish by the natives of Torres Straits in fishing for turtles (*Nature*, 17 January, p.285, 1889), I may call attention to the paper on this subject read by Frederick Holmwood, C. B., late H.B.M. Consul at Zanzibar, before the Zoological Society of London, on 17 June 1881, which Haddon does not seem to be acquainted with. Holmwood has fully described the mode of the use of the *Remora* by the native fisherman of Zanzibar in catching turtles and fishes. It is curious to find a somewhat similar method of employing the *Remora* practised by the islanders of Torres Straits.

P. L. SCLATER

From *Nature* **39**, 295; 24 January 1889.

Hexagonal polar current on Saturn

THE remarkable hexagonal feature surrounding Saturn's north pole apparent in the figure was discovered by D. A. Godfrey (*Icarus* 76, 335–356; 1988) while making polar projections of Voyager images. The outermost circle is at about 65° latitude and represents the edge of a zonally symmetric current in Saturn's atmosphere. At the time of the spacecraft fly-by in 1981 the northern hemisphere was entering spring and the polar region was sufficiently illuminated to show features, although great care was necessary to remove shading due to twilight effects. The mosaic figure, one of six presented (each showing the hexagon), is made up of four images taken of the sun-lit hemisphere as the planet rotated. Because Voyager's orbit kept it close to Saturn's equator, Godfrey had to reproject the foreshortened images of the north pole to produce the view shown, looking down the planetary axis. The morphology of the clouds does not change a great deal during one 10-hour rotation period, so that the mosaic is not very much different from an instantaneous snapshot. The four 'spokes' are boundaries between images, and the streaked appearance near the pole is an artefact of the projection.



D. A. Godfrey

Small differences between sequential mosaics can be used to determine the drift rate of clouds, and it is here that surprises emerge. The hexagonal feature is an atmospheric jet stream whose position and configuration remain fixed, to within a few metres per second, but the current in the jet exceeds 100 m s^{-1}. In this context, 'fixed' means stationary in latitude at about 76°, and in longitude relative to the periodicities which appear in radio emissions from the planet, presumably linked to the magnetic field which in turn is anchored to the deep core of the planet. Thus this is a jet stream which follows a twisting yet stationary path, rather than meandering, as is the familiar case in Earth's atmosphere and oceans.

Godfrey discusses the possibility of a direct connection between the hexagonal structure and the magnetic field, but concludes that it is improbable. The clouds are ammonia crystals at a level where the atmospheric pressure is about half an atmosphere and the temperature is about 120 K, well within the 'troposphere' (lower atmosphere) rather than the ionosphere. The conductivity is low and magnetohydrodynamic effects should not exist. Also unlikely is a direct connection between the radio emissions and the planetary weather, as these emissions do not have the character of lightning bursts. The simplest idea is that the magnetic field and the atmospheric current both have longitudinal structure that is of deep origin, and hence they show closely similar rotation rates.

Godfrey's kinky current is one more example on a growing list of stationary, or at any rate slowly moving, flow structures on the outer planets. Since the time of Galileo we have known of the Great Red Spot of Jupiter, which drifts in longitude with speeds of only a few metres per second, although it is composed of (and resides in) currents whose speeds reach 100 m s^{-1}. Smaller spots on Jupiter also tend to be slowly moving. T. E. Dowling and A. P. Ingersoll (*J. Atmos. Sci.* 45, 1380-1396; 1988) have shown that the circulation patterns near and within these spots can be used to infer the nature of flows at deeper levels, although they do not point to any particular reason for very slow motion of the spots. And there are slowly moving large-scale thermal features, discovered in the infrared by J. A. Magalhães *et al.* (to be published in next week's *Nature*) located at low latitudes on Jupiter, right where the broad equatorial current is also situated. The current is apparently perturbed by some influence that is nearly stationary.

All of these features could be providing information about deeper regions. Jupiter and Saturn have only small solid cores, unlikely to have any influence on surface motions. The vast bulk of these planets is fluid hydrogen and helium in roughly cosmic abundance. Heat emerges from the interiors in large quantities, comparable to the solar heating. The nature of the fluid motions which carry this heat to the surface has long perplexed astronomers and those studying convection. We may at last be seeing evidence, albeit indirect, for the morphology and flow speeds associated with this convection.

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Palaeontology

Fossil reptiles from ancient caves

Michael J. Benton

ONE of the problems of studying terrestrial fossil sites is that large plants and animals seem to be commoner than small ones, whereas the opposite pertains in nature. This could be because organisms were on average larger in the past; or because small ones are less likely to be preserved. The latter explanation seems to be the case as sites have been found, although only occasionally, which contain abundant small fossils. One such is the fossilized cave systems of south Wales and Bristol in the United Kingdom. In a new paper, N.C. Fraser[1] reports the discovery of small animals from an age where most sites reveal dinosaurs and other large animals.

The abundant small beasts of the Bristol–South Wales region offer a fascinating glimpse of life on land beneath the feet of the first dinosaurs. Despite their abundance and the good quality of preservation, the fauna is not perfectly fossilized. The skeletons are found at the bottom of fissures eroded into the ancient limestone hills. As the openings into the fissures are often small, the larger animals have effectively been filtered out. Nevertheless, the fissure animals provide unique

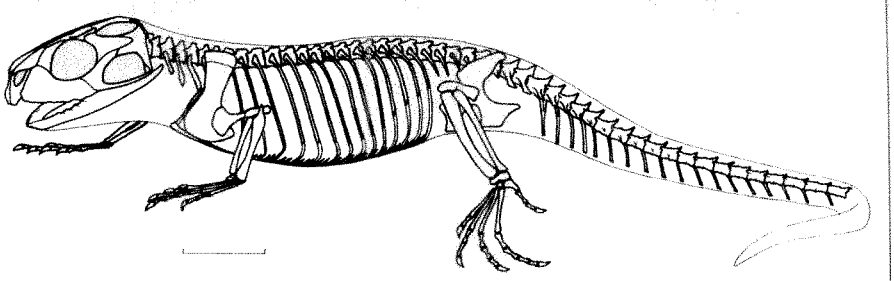


Fig. 1 Composite restoration of the entire skeleton of *C. hudsoni*. Bar, 2 cm. (From ref. 1.)

insights into the lives of the more abundant animals at this time of major faunal turnover. Some of the fissures were under water at the time of fossil formation.

The fossiliferous fissure deposits are dated as Late Triassic to Early Jurassic (about 220–200 million years old), the time during which the dinosaurs became established, and during which most 'modern' land vertebrates arose: mammals, turtles, crocodilians and lepidosaurs. These smaller 'modern' vertebrates are best represented in the fissures and they have yielded significant new phylogenetic information.

Fraser's find[1] of the sphenodontid *Clevosaurus hudsoni*[2] allows him to perform a detailed analysis of this animal and of its relationship with other organisms. *Clevosaurus*, a small 250-mm-long reptile, is superficially like a modern lizard, with a slender body, long limbs and (probably) a long tail (Fig. 1). The teeth are chisel-like and, unusually for a vertebrate, fused firmly to the bone of the jaws (Fig. 2). The teeth and jaw bones of adult specimens of *Clevosaurus* show signs of heavy wear, and the jaw action was a shearing one, rather like the action of a well-adjusted pair of scissors. *Clevosaurus* probably fed on hard-skinned invertebrates such as centipedes, insects and snails. It may also have been a facultative herbivore, raking plant food together with its beak-like front teeth, and chopping it further back in the mouth. Young *Clevosaurus* specimens have small, sharp teeth which may have been used for eating soft-bodied invertebrates such as worms.

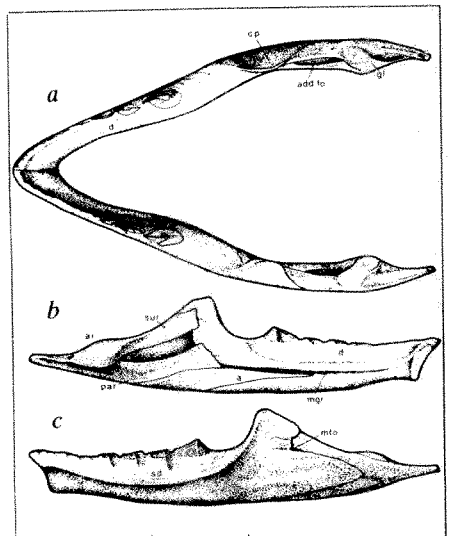


Fig. 2 Composite restoration of the mandible of *C. hudsoni* in: *a*, dorsal; *b*, medial; *c*, lateral views. Scale bar, 1 cm. (From ref. 1.)

Fraser[1] distinguishes two or more species in his material of *Clevosaurus*. The material is mainly disarticulated, and he examined more than 1,000 separate bones. Fortunately, he found a few more complete specimens which helped in the identification of isolated elements. The second *Clevosaurus* species, *C. minor*, is much smaller than *C. hudsoni*, reaching an estimated total length of 150–200 mm. They have adult patterns of tooth wear, so are unlikely to be juveniles of *C. hudsoni*. *C. minor* is found at different sites from *C. hudsoni*, so they are probably independent breeding species.

Other associated vertebrates from the Triassic fissures[3] include a gliding reptile, *Kuehneosaurus*; the early crocodilian *Terrestrisuchus*[4]; the dinosaur *Thecodontosaurus*[5]; the very early mammal *Kuehneotherium*[6]; various unidentified archosaurs[7]; and up to ten species of sphenodontid[1,8–10]. The Jurassic fissures contain a rather different fauna, consisting of the mammal *Eozostrodon*, the tritylodont *Oligokyphus*, the lepidosaur *Gephyrosaurus* and fish teeth[8].

The sphenodontids, such as *Clevosaurus* and its kin from the fissures, are of great phylogenetic importance. They are broadly similar to the living tuatara *Sphenodon* from New Zealand, a so-called living fossil. The fact that a complex fauna of up to ten sphenodontids co-existed in the south-west of Britain 220–210 million years ago suggests that the group was once as flourishing as the lizards are today[10]. □

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Daedalus

If I had a hammer

THE hammer is one of the most basic of implements. It exploits a simple newtonian principle: the deceleration of its head imposes a force on the object it strikes. In the hands of a skilled craftsman, it can crack a brick precisely in two, or make a controlled adjustment of 0.01 millimetres.

In the hands of unskilled amateurs, however, it produces a vast global total of bent nails, bruised thumbs, dented woodwork and smashed panes of glass. Daedalus is therefore updating this palaeolithic invention with the most advanced electronics. He has been inspired by the modern thyristor photographic flash unit. This integrates the light reflected off the scene and, when enough has been received for a good exposure, quenches the flash. It all happens in microseconds. In the same way, Daedalus's electronic hammer carries an accelerometer which integrates the momentum received by the target during the impact. When this exactly reaches a pre-set value, the impact is 'switched off'. A fast-acting trigger mechanism snatches back the hammer head and dumps the surplus momentum back into the handle and the user's arm.

This wonderful tool not only tames and directs the enthusiasm of the user: it educates him too. Set for a delicate task (such as tapping a ceramic tile into place on its mortar) it will transmute a wild swipe into a light and precise blow. But the excess momentum, returned to the user's arm as an unexpected 'kick', warns him that his blow was too heavy. His next swipe will not be so wild. Soon he will have a sound intuitive feeling for the exact use of the hammer in this task. Professionals and amateurs alike will welcome the electronic hammer: in all fields of engineering and domestic construction it will teach the subtle kinetic intuition of the true craftsman. Indeed, electronically controlled impact may become the preferred way of carrying out all sorts of tasks now conducted manually, from removing a stopper to advancing a pawn or shaping a cake. Daedalus is devising a 'personal electronic hammer' to fit in the pocket like a pen. The user will turn to it for any manual task as automatically as he reaches for a pen to write with, or a calculator to do arithmetic.

But the best strategy for developing a new invention is to launch a high-value product first, on whose profits the mass market can be attacked. So Daedalus's first commercial product will improve the most valuable impacts of them all — those of international golf. His electronic golf clubs, bringing calibrated exactitude to this most obsessive and unforgiving of games, should sell for tens of thousands of pounds.

David Jones

Setting radiation standards

SIR—The setting of standards of protection against ionizing radiation, already controversial, is being confused by the difficulty of specifying clearly the significance of the risk to an exposed individual. 'Risk' is used here to mean the probability of a deleterious event. The term 'detriment' can be used to combine the probability and the severity of that event.

For many years, the fatal risk to an individual has been presented as the probability of attributable death with no allowance for competing causes of death. The effect of this allowance is known, but has not been used explicitly in setting standards. The effect of latency on the degree of detriment has also been neglected, although a death in later years is less detrimental than an earlier one.

More recently, in its 1988 report to the UN General Assembly[1], the United Nations Scientific Committee on the Effects of Atomic Radiations (UNSCEAR) has addressed both these issues. The allowance for competing causes of death is explicitly included in the risk estimates, and the effect of latency on the detriment is indicated by estimating the average years of life lost. Information on the distribution of risk with time after exposure is still incomplete because many members of the exposed populations being studied are still alive. UNSCEAR offers two risk-projection models, one with the risk constant (after a latent period) — an additive model — and the other with the risk increasing with time as does the risk of 'natural' cancer — a multiplicative model. The letter predicts about twice as many deaths as the former with no more years of lost life. Unfortunately, most commentators on this topic have stressed the increase in fatal risk without considering the decrease in years lost per attributable death.

There is a further weakness in using risk alone. The total risk of death cannot be increased; it is unity. Radiation can change the time and cause of death, not the overall probability.

The risk to the individual is thus a poor measure of the consequences of exposure to ionizing radiation. We need a multi-attribute approach to take account of the time and nature of a death in addition to its probability. This would also make it easier to include explicitly the non-fatal effects such as curable cancer and hereditary defects. It is to be hoped that those reponsible for setting standards, particularly dose limits, will not rush into changes based only on the simple concept of risk. As both the International Commission on Radiological Protection[2] and the National Radiological Protection Board[3] have indicated, the most appropriate immediate reaction is to increase the attention paid to the need to keep all exposures as low as can reasonably be achieved. Quantitative changes in standards should be a more measured response.

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X-chromosome reactivation and ageing

SIR—At first sight it seems that the results of Migeon *et al.*[1] on the low reactivation frequency of the human X-linked hypoxanthine phosphoribosyl transferase (HPRT) gene do not confirm the earlier finding of the strong age-related reactivation of the mouse X-linked ornithine carbamoyl transferase gene (OCT)[2]. Whereas the spontaneous reactivation of the silent OCT gene was increased 50-fold in mice older than one year, the reactivation of the silent human HPRT gene in female Lesch–Nyhan heterozygotes is rare at any age.

In studies of the process of ageing, one of the main challenges is to understand how similar physiological or biochemical events occur at very different rates in species with different lifespans. For example the crosslinking of collagen is known to increase with age, but although the chemistry is the same, the rates of reaction are very different in different species[3]. The same is true for the accumulation of the age pigment lipofuscin, the denaturation of lens crystallin and other 'biomarkers' of ageing, as well as many pathologies, including the appearance of neoplasms. It is well known that mouse cells transform to malignant cells readily in culture, whereas human cells are very refractory to this transformation. This makes biological sense, because on a per cell basis, small short-lived animals have a far higher incidence of cancer than large long-lived ones.

It is therefore reasonable to argue that the age-related reactivation of a silent X-linked gene would be many times higher in mouse cells than in human cells. Another way of saying the same thing is that the controls of cellular metabolism, or cellular homoeostasis, must be very much tighter in long-lived animals than in short-lived ones, and this would of course include the silencing of gene expression by DNA methylation[4]. It is already known that the maintenance of cytosine methylation in DNA in primary diploid cells is much more effective in human than in mouse cells, and that hamster cells have an intermediate level of maintenance[5].

Two of the results reported by Migeon *et al.* together indicate that there may be a low loss of methylation in normal human cells with ageing.

First, there is a slight increase with age in the proportion of cells with active HPRT, which is an insensitive assay, and the results are of marginal statistical significance ($P=0.08$).

Second, using a much more sensitive assay, it is shown that the silent HPRT gene can be more easily reactivated by the demethylating agent deoxyazacytidine in cells from old individuals than in cells from young ones ($P<0.005$).

These results would be expected if the methylation of many sites in the CpG-rich island of the human HPRT gene declines slowly with age, but the overall silencing mechanism is fairly efficiently maintained. However, a gradual loss of methylation would increase the probability of reactivation by a demethylating agent. If this interpretation is correct, then similar experiments carried out with female 'Lesch–Nyhan' heterozygous mice[6,7], should show a significant age-related reactivation of HPRT. Such a result would reinforce the view that cellular homoeostasis is much better maintained in long-lived species than in short-lived ones[4,8].

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Misidentified cell

SIR—Because the cell line TE671 is said to originate from an early culture of a human medulloblastoma[1], it has been widely used to investigate this tumour type and as a model for primitive neural cells in general. And because the cell line can be infected by the AIDS virus HIV[2] (in a way that cannot be blocked by soluble CD4, as reported on page 368 of this issue[3]), TE671 could provide an ostensibly useful *in vitro* model of HIV infection of the brain. Our evidence, however, suggests that TE671 is not a neural cell but a muscle cell.

We note that TE671 cells have a number of features in common with the rhabdomyosarcoma cell line RD. They have several of the phenotypic characteristics of striated muscle cells, including the presence of desmin, myoglobin[4] and the

muscle-type acetylcholine receptor[5]. We have found that both cell lines contain an N-*ras* gene that is activated by a point mutation at the third base of codon 61 and, in new cell stocks obtained directly from the American Type Culture Collection, that the majority of marker chromosomes are common to the cell lines. Furthermore, DNA fingerprinting with locus-specific and core probes fails to reveal differences between the two lines.

Both cell lines were derived in the same laboratory but RD was described[6] and submitted to the ATCC before the first report of TE671. When considered together these observations indicate that TE671 is most probably a subline of the rhabdomyosarcoma cell line RD.

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Spectral peaks

SIR—Lutz and Watson[1] discuss the frequency spectrum of the Harland *et al.*[2] geomagnetic reversal record of the past 165 million years. This spectrum has been interpreted as showing a 30-million-year short-term oscillation superimposed on an aperiodic long-term variation. Lutz and Watson argue that the long-term component, $\lambda_L(t)$, dominates the frequency spectrum for periods P up to 40 million years. Here I show that the spectrum in this range is essentially determined by the strong boundary discontinuities introduced by performing a Fourier transform $\lambda_L(t)$ over a finite interval (τ_1, τ_2); that is, these discontinuities dominate over any time variation of $\lambda_L'(t)$ between τ_1 and τ_2.

The influence of discontinuities becomes apparent when the limits of the Fourier transform are extended to $(-\infty, +\infty)$. This leaves all integrals unchanged, provided that the 'extended' function $\lambda_L'(t)$ has the form

$$\lambda_L'(t) = \begin{cases} \lambda_L(t) \text{ for } \tau_1 \leq t \leq \tau_2 \\ 0 \text{ otherwise} \end{cases} \quad (1)$$

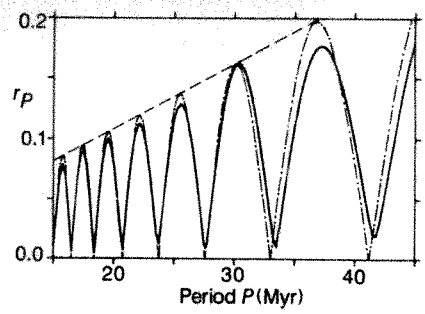
As $\lambda_L(t)$ is largest at the boundaries, $\lambda_L(t)$ exhibits large discontinuities there. The dominance of these discontinuities can be demonstrated by considering a simplified trial function

$$\lambda_L^0(t) = \begin{cases} \lambda_d = \text{constant for } -T/2 \leq t \leq T/2 \\ 0 \text{ otherwise} \end{cases} \quad (2)$$

which retains the two boundary discontinuities (of size λ_d, taken to have the value 5 Myr^{-1}, and separation $T = \tau_2 - \tau_1$) but carries no information on variations in between. The spectrum of this function,

$$R_P^0(T) = N^{-1}\lambda_d (P/\pi) \left| \sin(\pi T/P) \right| \quad (3)$$

is compared in the figure with that of $\lambda_L(t)$ obtained by Lutz and Watson[1]. (Here N is the total number of reversals.) We interpret the close correspondence in shape, position and peak heights as verification of our initial claim. The straight dashed line denotes the exact small-P asymptotic envelope for any function $\lambda(t)$ that has the same discontinuities as $\lambda_L'(t)$ and $\lambda_L^0(t)$. The positions of the maxima of $R_P^0(T)$ are given by $P_n \approx T/(n+1/2)$ for integers $n \geq 0$; for all T, they agree well with the values from the numerical Fourier transform of $\lambda_L(t)$ given in Table 1 of ref. 1, for which τ_1 was varied in discrete steps. Therefore, the grouping together there of peak positions with more or less constant period is arbitrary. When $\tau_2 - \tau_1$ decreases continuously, the peak positions do so too.



Fourier spectra of two long-term reversal functions plotted against period $P = 2\pi/\omega$. $R_P^1(\tau_1, \tau_2)$, from ref. 1, is shown as a solid curve, and $R_P^0(T)$ (equation (3)) as a dash-dotted line. The straight dashed line is the exact asymptotic envelope for $P << T$ to both spectra.

The influence of short-term oscillations would thus be seen only as noticeable deviations of the peak heights from those of $R_P^0(T)$, that is, from the envelope of R_P (τ_1, τ_2). Using a Poisson process, Lutz and Watson[1] show that the long-term component alone may generate fluctuations in the peak heights that are large enough to account for the spectrum of the total reversal function $\lambda(t)$.

Thus, for periods $\lesssim 40$ Myr, the spectrum R_P (τ_1, τ_2) of the total reversal function is determined mainly by the strong discontinuities in $\lambda_L(t)$ at τ_1 and τ_2, and hides any information on short-term variations. These can be reliably detected in the spectrum only if the discontinuous long-term variation can be subtracted before the Fourier transform operation.

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RNP in maize protein

SIR—We wish to point out an interesting feature in the sequence of the abscisic acid-induced glycine-rich protein (AAIP) of maize recently reported by Goméz *et al.*[1]. The protein contains a sequence (see below) which conforms perfectly to the RNP consensus sequence[2-4] that has been found so far in heterogenous nuclear RNA-, messenger RNA-, pre-ribosomal RNA-, and small-nuclear RNA-binding proteins.

Maize AAIP:

$\cdots$ Arg^{49} Gly Phe Gly Phe Val Thr Phe^{56}...

RNP consensus sequence:

... Lys Gly Phe Gly Phe Val Thr Phe...
Arg Tyr Ala Tyr X Tyr

This strongly suggests that AAIP is a single-stranded nucleic-acid binding protein, most probably an RNA-binding protein (RNP). Several other features of the AAIP sequence also resemble those found in some of these proteins. The protein appears to contain two distinct domains: an amino-terminal domain (about 90 amino acids) that contains the RNP consensus sequence, and a carboxy-terminal glycine-rich domain. The amino-terminal domain is the putative RNA-binding domain[4]. There are extensive similarities in the general character of the amino-terminal domain with that of the putative RNA-binding domain of several RNP proteins[4]. A glycine-rich domain is also a feature of some RNP proteins (for example, the hnRNP protein A1 (ref. 5), and the nucleolar protein nucleolin[6]). AAIP may be the first plant protein described so far to contain an RNP consensus sequence. The predicted property of AAIP as an RNA-binding protein can be readily tested, for example, by chromatography on small-subunit DNA columns, on ribohomopolymer columns, and by photochemical crosslinking to RNA.

Abscisic acid is a hormone that appears to modulate the response of plants to adverse conditions, and it seems to also play a role in embryogenesis. Determining if AAIP is indeed an RNA-binding protein, and if so, what RNA it binds to should be important for understanding the mechanism of these important processes.

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Molecular Biology and Cell Biology,
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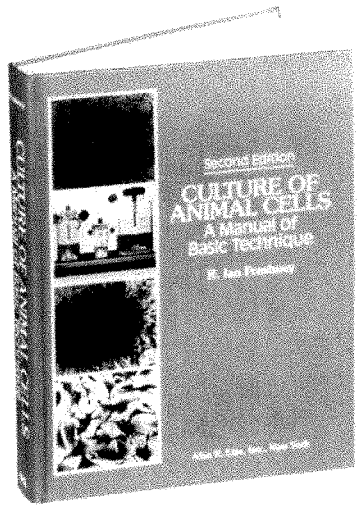
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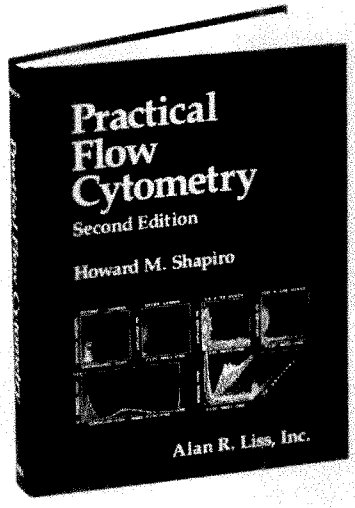
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Prized developmentalist

Tim Hunt

The Heritage of Experimental Embryology: Hans Spemann and the Organizer. By Viktor Hamburger. *Oxford University Press: 1988. Pp. 196. £22.50, $29.95.*

VIKTOR Hamburger, as old as the century, was a graduate student in Hans Spemann's laboratory in Freiburg. He was an exact contemporary of Hilde Mangold (*née* Proescholdt), who performed the famous 'organizer experiment' (see figure) that clinched Spemann's reputation and won him the only Nobel prize ever awarded for developmental biology. Over 30 years later, in St Louis, Missouri, Hamburger was head of the laboratory in which nerve growth factor and epidermal growth factor were discovered by Rita Levi-Montalcini and Stanley Cohen, work that in turn won them a Nobel prize. It is clear from Levi-Montalcini's account of this period that Hamburger was all that a boss should ever be, encouraging, supportive, insightful and critical, and only his perfect modesty explains why he is not as celebrated as his erstwhile mentor or his own pupils.

Hamburger's account of Spemann and the organizer is a masterpiece. "No subject in embryology has been so misrepresented and misunderstood as the properties of the organiser" according to Jonathan Slack[1], but Hamburger does a superb job of explaining the experiments, setting them in historical context and tracing their subsequent experimental and analytical history. Anyone with the faintest interest in developmental biology should read this book. It is an eye-opening inspiration.

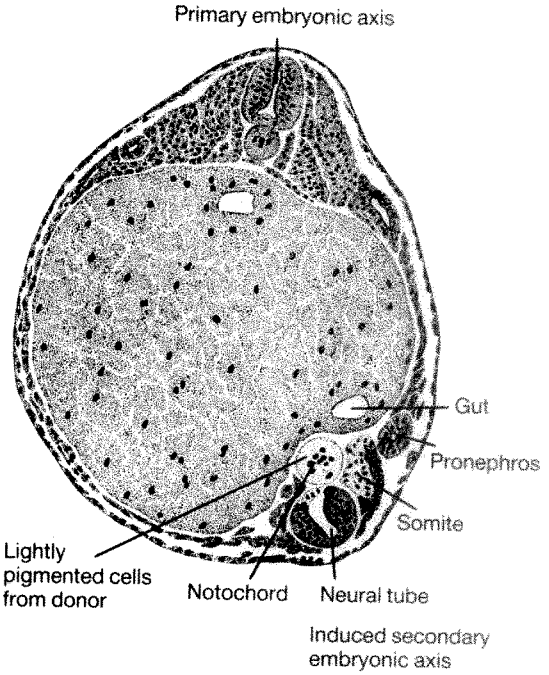
The purely scientific account is enlivened by biographical sketches of the protagonists, done with graceful affection and respect. An appendix describes Hilde Mangold and brilliantly evokes student life in Freiburg in the early 1920s. We discover that the PhD students actually had to know some philosophy, attending lectures by Husserl and Heidegger. The political climate was violent, the inflation appalling, yet the arts flourished and the mountains frequently called the young away to relax; no wonder such great work was done. And although they "cared more for food for thought than nourishment for our bodies", the dreadful "turnip winter" put them off that vegetable for life.

Hamburger was close to Hilde Mangold, who alas died in 1924 from burns sustained when a petrol heater exploded at home. She had not been pleased when Spemann insisted on putting his name on her paper (and first at that); but according to Hamburger, the design and conception of the experiments were Spemann's and Mangold was the fortunate student assigned to carry them out. She died before her work became widely appreciated.

Hamburger analyses these classical experiments and ideas in developmental biology as clearly as I have ever seen it done, and traces Spemann's scientific legacy almost to the present day. He points out errors, ambiguities and misconceptions as well as describing the technical problems that beset the early workers. Spemann, it turns out, did not appreciate the importance of the ionic composition of the medium for the survival of his embryos (at one time he had to send back to Würzburg for water to make things work; calcium was probably the missing ingredient). It was Johannes Holtfreter who discovered the importance of this and of the need for scrupulous sterility, which allowed him to perform much more sophisticated experiments. Nowadays, antibiotics assure the embryos' well-being, and advances in reproductive physiology mean that experiments do not have to attend on the natural breeding season of the animals.

Nevertheless, Spemann was a great craftsman. His use of baby's hair for the 1904 constriction experiments is legendary, and he invented all sorts of other useful tools for cutting and transplanting bits of the tiny embryos. Moreover, his analytical acumen matched his experimental powers, and though he confessed to a preference for "psychical" rather than physical metaphors in the analysis of his data[2], he was never carried away by more abstract theory of the kind that led Driesch ultimately to espouse vitalism. In his imagination Spemann was at one with the objects of his study, which allowed him and his followers to extract the maximum of clear information from their transplantation experiments. As Walther Vogt, the great fate-mapper, explained at Spemann's sixtieth-birthday celebration: "The result is not sought by theorizing, thus passing over the object; nor by treating the embryo as a pure object, but rather by questioning it like a living partner, in a dialogue".

Spemann was no chemist, nor yet a physiologist (as the story of the water makes clear), and only towards the end of his career did the search for a chemical embodiment of the organizer begin, with mixed results. Spemann had regarded the inductions he studied as properties of whole tissues, and barely even considered organization at the cellular level. What had impressed him was that an almost complete second embryo could be induced simply by transplanting a tiny sliver of tissue from one embryo into another. This is curiously holistic biology,



The organizer experiment — Spemann summarized the experiment as "The astounding fact that a secondary embryo may be brought about by transplantation". The upper edge of a blastopore from a lightly pigmented newt gastrula was transplanted to the side of a gastrula of a different species of newt with dark pigmentation, giving a gastrula with two blastopores. The graft 'took' and invaginated at its new site. The resulting embryo had two neural plates, two sets of somites, two guts and so on as can be seen in the cross-section shown here. The pigmentation marker showed that the secondary embryo was composed of cells derived from both donor and recipient tissues. Hence the implant was said to 'organize' a secondary embryonic axis running along the flank of the recipient. This property is confined to the upper edge of the blastopore. It is perhaps the most striking known example of embryonic induction. The illustration, which has been relabelled, is taken from Embryonic Development and Induction, *by Hans Spemann (Yale University Press, 1938).*

yet meticulously and rigorously dissected on its own terms, as Hamburger makes plain.

What is fascinating and puzzling about this period as seen with modern eyes is the apparently complete lack of communication between geneticists and developmental biologists. T.H. Morgan and his colleagues had laid out the basis of modern genetics by the end of the First World War, and Morgan himself was familiar with the main issues in developmental biology through working in marine stations at Naples and Woods Hole. Yet in his 766-page book *Experimental Embryology* (1927), Morgan devoted a scant four pages to the role of chromosomes in development. Too little was known — "Whether all the genes are active all the time, or whether some of them are more active at certain stages of development than are others, are questions of profound interest". Nor was it then certain whether the chromosomes were "concerned with the making of enzymes". Morgan also devoted considerably less space to embryonic induction than he did to parthenogenesis, but that is another story.

I would be curious to know whether Spemann and his school ever thought about how genes might shape the tissues, or what kind of form they imagined a complete account of embryonic induction would eventually take. Of course, in the mid-1920s there was little enough known about proteins and enzymes, let alone how genes might control their production. Given this ignorance, it is amazing how far the analysis of determination, differentiation and induction could progress, and a revelation to modern molecular eyes to see how much there is still to explain. My only regret about Hamburger's book is that it stops short of the cloning revolution, and sticks closely to amphibian development. It would be marvellous to hear his views on induction, fields and gradients in their modern dress. □

1. Slack, J.M.W. *From Egg to Embryo* p.53 (Cambridge University Press, 1983).
2. Spemann, H. *Embryonic Development and Induction* pp.371–372 (Yale University Press, 1938).

Tim Hunt is a Lecturer in the Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK.

Condensed matter

W. R. A. Brown

Heterochromatin: Molecular and Structural Aspects. Edited by Ram S. Verma. *Cambridge University Press:1988. Pp.301. £30, $49.50.*

AT THE end of a eukaryotic mitosis, most of the chromatin in the cell decondenses. Some does not and remains genetically inactive — this material, with its distinctive cytological properties, is called heterochromatin.

Until recently there has been a gap between the chromosomal features that can be described with the help of a microscope and the size of DNA amenable to molecular analysis. Now, however, pulsed-field gel electrophoresis and molecular cloning in artificial yeast chromosomes are making it possible to analyse megabase pair stretches of DNA, and thus to develop molecular maps of entire eukaryotic chromosomes. These maps will include the structural information necessary for analysis of eukaryotic chromosome behaviour at a molecular level, a prospect which has brought back to prominence an older, sometimes arcane, cytogenetic literature which depicts a eukaryotic chromosome biology of extraordinary diversity. A further consequence has been the resurgence of interest in a variety of dormant cytogenetical questions, such as those surrounding the functional significance of constitutive heterochromatin.

Heterochromatin is a multi-author volume which sets out to give a contemporary overview of the subject. Appearing as it does at this singular time, it is likely to attract considerable attention. Sadly, in my view, most readers will find it a disappointment.

The first chapter, "The Biology of Heterochromatin" by B. John, occupies nearly half of the book and is an account of the cytogenetics of heterochromatin in animals. Position-effect variegation, segregation distortion, ectopic pairing, heterochromatin elimination and more are reviewed critically. If you need a guide to the older cytogenetic literature, then this is it.

The remaining six contributions can be considered in three groups. A. Lohe and P. Roberts review the sequence organization of satellite DNA in *Drosophila melanogaster* and other *Drosophila* species; this chapter will be of particular value to those working either on other aspects of *Drosophila* biology or on the satellite DNA of other organisms. The next three chapters describe aspects of our knowledge of the ultrastructure of heterochromatin. Here there is duplication and unresolved contradiction, and it would have been better to have had this interesting and apparently controversial area covered in a single authoritative article. The last two contributions describe two rather specialized aspects of human cytogenetics, the first dealing with the use of restriction endonucleases as reagents to help produce banding patterns in human chromosomes, the second with the techniques for detecting human chromosome heteromorphisms.

Altogether this is an uneven book, both in organization and in the quality of the contents. Much is omitted. The discussion of satellite DNA in *Drosophila* is useful, but why is there no general review of tandemly repeated, simple-sequence DNA in other organisms? The molecular basis of mammalian X-chromosome inactivation is covered only briefly. Heterochromatin in plants is mentioned only in passing. Thus the editor has failed in his goal of putting together a comprehensive account of the subject. Nevertheless John's review alone justifies publication of the book, and will ensure that it has a part in the revitalization of the study of heterochromatin. □

W. R. A. Brown is in the Cancer Research Campaign Chromosome Molecular Biology Group, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK.



Swooping down the ages — a flying machine, made of natural materials, and based on drawings of Leonardo da Vinci. The machine features in the Leonardo exhibition which opens at the Hayward Gallery, London, today. The book-of-the-exhibition ("an invaluable guide to Leonardo's universe") is also published today by Yale University Press.

Volumes of human learning on machine learning

Max Bramer

Proceedings of the Fifth International Conference on Machine Learning. Edited by John Laird. *Morgan Kaufmann: 1988. Pp. 467. Pbk $24.95. In Britain distributed by Afterhurst, 116 Pentonville Road, London NW1 9JB, £19.95.*

EWSL 88: Proceedings of the Third European Working Session on Learning. Edited by Derek Sleeman. *Pitman: 1988. Pp. 263. Pbk £25.*

ARTIFICIAL intelligence (AI) is the branch of computer science concerned with developing systems with sophisticated reasoning capabilities — capabilities which, if possessed by human beings, would be described as intelligent. A decade ago AI was seldom heard of outside the research laboratory or the realms of science fiction. The turning point came in 1981 with the setting up in Japan of the ten-year programme to build 'fifth generation' computers for the 1990s, in which "intelligence will be greatly improved to approach that of a human being".

Although it may have seemed a heretical idea at that time, AI systems (for problem solving, computer vision, natural-language processing and other applications) are becoming part of the mainstream of computing. There are now hundreds (maybe thousands) of so-called expert systems for practical use in medicine, geology, chemistry and, increasingly, finance. Essentially, the construction of an expert system involves the elicitation of knowledge from a subject expert and its representation in a computer program, often in the form of rules. Obtaining such knowledge is often a time-consuming and error-prone activity, however, and increasingly attention has turned to finding ways in which this process may be automated. Such considerations provide one — although by no means the only — motivation for the recent resurgence of interest in machine learning.

Human intelligence

Although the exact nature of human intelligence remains unclear (and is likely to do so for a long time), the ability to learn is undoubtedly a crucial element, and a multi-faceted one. To list only some possibilities, human learning can be by rote, by trial and error, by discovery, by generalizing from a series of example cases or by a detailed analysis of a single specific case. Humans can learn by self-instruction or by being taught. Teaching can take the form of imparting an underlying theory and/or showing examples of good (or bad) practice.

Much human learning takes place by generalizing from a series of examples. There are many ways in which this can occur: the examples can be presented in a carefully chosen sequence by a teacher, can be chosen at random or in some situations can be self-selecting (for example, patients attending a doctor's surgery). The learner can examine a collection of examples as a whole to develop a strategy for all future cases, or can begin with a simple strategy which is then refined incrementally from further examples as he or she goes along. These and other aspects (such as inductive reasoning) of the human skill known as learning are reflected in the lines of research currently being pursued in machine learning.

Two forms of machine-learning research for which the link with human behaviour is less apparent are 'connectionist' and 'genetic' learning. Connectionist learning is closely related to work on neural nets, a field which has become a bandwagon in the past year or so. Although some interesting results have emerged, it is difficult to evaluate the work concerned without considering the very strong claims which many of its proponents have made about modelling the physiological workings of the human brain. Such claims are notoriously easy to make, but much more difficult to justify. I myself remain unconvinced by them.

The term 'genetic' learning originates from the observation that natural (biological) selection can be regarded as a form of learning, where a simple yet powerful learning strategy is applied repeatedly to a continuous stream of input, whilst the system moves from a rudimentary state (such as a simple single-cell organism) to increasingly more effective ones (such as *Homo sapiens*). In machine learning, genetic algorithms embody some model of natural selection and genetics as their principal learning mechanism. Such algorithms have successfully been applied to tasks like the evaluation of the worth of a position in a game of checkers. Unfortunately, as with connectionist learning, the systems that result are likely to be inscrutable to people, however well they perform their given tasks.

The books under review are the proceedings of two conferences which took place in 1988. That edited by John Laird contains papers presented at the University of Michigan in June; 49 contributions are included, the overall stress being on theory rather than applications.

The way that conference proceedings are broken down into sections is often a good indicator of the balance of research in a particular field at a particular time. In this case, there are 15 papers on empirical learning, seven on explanation-based learning, one on case-based learning, five on machine discovery, four on connectionist learning, five on genetic learning, three on integrating explanation-based and empirical learning, and nine on other topics. Of particular interest are a number of contributions on the well-known ID3 algorithm for constructing decision trees from large collections of examples, a technique which has been strongly advocated as a powerful way for developing the decision rules to use in expert systems.

Goals and experiments

The EWSL proceedings, which come from a meeting held in Glasgow in October, contain 21 papers. Unfortunately they have not been grouped into sections and have simply been arranged in alphabetical order of author, thus losing whatever structure they had when presented at the conference. As a result two valuable, general articles are buried in the middle of the text when they could usefully have been placed at the beginning as an introduction. These are Donald Michie's "Machine Learning in the Next Five Years", in which he considers the past, present and future of the subject, and the paper by Dennis Kibler and Pat Langley on "Machine Learning as an Experimental Science", in which the authors take a broad look at the goals that underlie the experimental study of learning systems and the types of experiments necessary to achieve them.

Both books will be required reading for those seriously involved in machine-learning research, and will no doubt be frequently cited in the future. Despite its lack of structure, the EWSL volume is the more accessible and perhaps the more valuable collection. But both suffer from the failings of most such volumes — the papers generally lack the broad context necessary to be meaningful to the general reader, or in many cases the researcher in an adjacent part of the field. At a more mundane level, neither book has a subject index, and there are no cumulative lists of references.

These faults may well be unavoidable in books which are put together quickly, so that they can be handed out at a particular conference. But it is surely not asking too much that they should be remedied before the volumes are sold to a wider technical readership. It may seem harsh to criticize the publishers for a sin which is increasingly widespread in the AI world, and doubtless elsewhere. But the lack of some post-conference editing has undoubtedly resulted in two books which are less valuable (if more timely) than they otherwise might have been. □

Max Bramer is Manager of the Knowledge Engineering Programme, Hewlett-Packard Laboratories, Filton Road, Stoke Gifford, Bristol BS12 6QZ, UK, and Chairman of the British Computer Society's Specialist Group on Expert Systems.

Explanation and definition

Alan Isaacs

The Harper Dictionary of Science in Everyday Language. By Herman Schneider and Leo Schneider. *Harper & Row: 1988. Pp. 309. $25.*

The American Heritage Dictionary of Science. Edited by Robert K. Barnhart. *Houghton Mifflin: 1988. Pp. 740. $19.95.*

THESE two books, both called dictionaries of science, are about as dissimilar as any two books claiming to be dictionaries of anything could be. The essential difference is one of the intended audiences.

The title of the Harper book is plain enough, and the blurb tells us that here is "a book that explains scientific terms and techniques with wit and charm and, most of all, in plain English". Although one may find it hard to imagine anyone being either witty or charming in scientific explanation, the Schneiders have been fairly successful in making fairly difficult concepts fairly lucid to fairly intelligent non-scientists. Interferometry, for example, is quite ingeniously explained, so too are computerized axial tomography and boolean algebra. Indeed, there are some extremely good explanations throughout the book, which one could well imagine being helpful to any layman willing to put up with the wit and charm that go with them.

The authors say (in the preface) that their dictionary came into being because a neighbour was confused by standard definitions of entropy — that the only people capable of understanding these definitions would be scientists. So their own entry begins wittily with T.S. Eliot's "This is the way the world ends..." and launches off charmingly into a piece about "the majestic rotation of galaxies". They do not record how their neighbour reacted; one cannot help wondering if he was much the wiser. At least it is clear from the entry — explanation without definition — at whom it is aimed.

Who, though, will use the American Heritage dictionary? On the inside flap of the jacket it is asserted that the book "bridges the gap between the oversimplified and the highly technical reference", while in his preface the editor notes that it is designed to "support" the student. One hopes he means that the biological entries are designed to help the physical scientist, and vice versa? If that is the case, the book could well serve a useful purpose. But a glance at the entry for entropy is not very reassuring if he doesn't mean this. The definition eschews explanation: "a measure of the unavailability of thermal energy for doing mechanical work", a not very helpful quotation from L.K. Runnels, and that is all. Surely, the student of physical science would want something more than that. Some attempt at explanation on the lines of $\Delta S = \Delta Q/T$, at the very least.

Another surprising aspect of Barnhart's book is the treatment of chemistry, which is both inconsistent and out of date. For example, there is no entry for ethanal and that for acetaldehyde does not even refer to this systematic name. There is, however, an entry for ethanol, which is given as an "also called" under ethyl alcohol. Ethene has an entry cross-referring it to ethylene, but the entry at ethylene makes no mention of ethene. In view of the international change from trivial to systematic names, it should be one of the prime tasks for a dictionary at this level to deal correctly with the relationship between the two systems, especially if it is for school and college libraries.

That neither dictionary has solved the problem of either explaining or defining entropy for the layman, the life scientist or the physical scientist, may not be a total condemnation of either. It *is* a hard concept to get across in any depth without mathematics. Moreover, both books contain plenty of other useful explanations (Harper) and definitions (American Heritage). □

Alan Isaacs is Managing Director of Market House Books, Market Square, Aylesbury, Buckinghamshire HP20 1TN, UK. Among other books he is editor of the Macmillan Encyclopedia *and the* Penguin Dictionary of Science.

Cycling essays

Edward Rybicki

Portraits of Viruses: A History of Virology. Edited by F. Fenner and A. Gibbs. *Karger: 1988. Pp.344. SwFr. 147, £66.90, $98.*

SINCE I began teaching virology in 1981, I have come across only one book that purported to be a history of the subject — and that is now sadly out of date. It was pleasing, therefore, to hear that Karger were to bring out the volume under review. My enthusiasm dwindled somewhat when I realized that the book consisted of the 15 "Portraits of Viruses" that have been published in *Intervirology* over the years since 1979, and that I had already read most of them. The essays were written by distinguished and long-serving virologists, and are often as much personal scientific biographies (especially those by Heinz Fraenkel-Conrat on tobacco mosaic virus, and Richard Lister on tobacco rattle virus) as they are contributions to the history of research on a particular group of viruses.

In the preface, the editors say that "this collection of essays has some notable gaps". This is very true: there are no reoviruses here, nor any polyoma- or adenoviruses, and there is only one retrovirus. Worse, the portraits have not been updated or expanded; all of them have been reproduced directly from the journal. The editors say that "for contributions written several years ago, we asked the authors to provide a brief addendum . . .". In fact, only the portraits of the poxviruses (F. Fenner, 1979) and the picornaviruses (J. Melnick, 1983) have such an addendum. The failure to bring the articles up to date is more excusable when their authors have since died — as have Macfarlane Burnet (influenza virus A), Peter Wildy (herpesviruses) and Basil Kassanis (tobacco necrosis virus and its satellite virus) — but it was remiss of the editors not to have insisted that contributors still living should do so.

The most recent essays were published in *Intervirology* in 1986; seven of the others appeared between 1979 and 1981. Thus it is that William Hayes does not include the exciting developments of the past few years, such as the sequencing of lambda and its development as a powerful tool in the recombinant-DNA armoury; Fraenkel-Conrat neglects the sequencing of the tobacco-mosaic-virus genome; in Burnet's essay there is no mention of the cDNA cloning and sequencing of many influenza virus HA and NA genes, nor of the synthetic peptide and monoclonal antibody work on the unveiling of antigen drift; and T. O. Diener does not tell of the elucidation of the replication and self-cleavage mechanisms of viroids. The essays themselves vary from the very personal and relatively sketchy, to the more formal and comprehensive review-type treatments given to poxviruses, picornaviruses and especially to Rous sarcoma virus (J. Svoboda): these have already proved helpful in my teaching, and remain as valuable references; I shall not be referring much to the other chapters.

These points apart, I have no complaints about the "Portraits of Viruses" series. But I do question whether it was necessary to put together this collection, or whether it will be worthwhile (the editors' hopes notwithstanding) to do so again in the future. The series of portraits always was, and remains, at best fragmentary and anecdotal. Publication of the essays in *Intervirology* is sufficient, in my view, to safeguard their authors' contributions to the body of background material which, one hopes, will be used by others for the writing of fuller accounts of the history of virology. □

Edward Rybicki is a Senior Lecturer in the Department of Microbiology, University of Cape Town, PB Rondebosch 7700, South Africa.

Keep Abreast of New Developments in Medicine

Oncology

Z. Tang, M. Wu, Shanghai; **S. Xia,** Wuhan (Eds.)

Primary Liver Cancer

Associate Editor: S. Ye

Foreword by L. Li and F. Qui

1989. 261 figures, 196 tables. Approx. 520 pages. Hard cover DM 460,–.
ISBN 3-540-50228-9
Distribution rights for People's Republic of China: China Academic Publishers, Beijing

Primary liver cancer (PLC) is one of the most frequent malignancies in southeast Asia and ranks third among all malignancies in China. Etiology, epidemiology, pathology, and early diagnosis at a subclinical level are examined by well-known Chinese experts.

UICC: International Union Against Cancer

I. T. Magrath, Bethesda (Ed.)

New Directions in Cancer Treatment

1989. 71 figures. XVIII, 634 pages. Soft cover DM 148,–. ISBN 3-540-19063-5

Contents: New Directions in Cancer Treatment: An Overview. – Physical Approaches to Therapy. – Chemotherapy. – Biological Approaches to Therapy. – Potential Approaches. – Therapeutic Trends in Specific Neoplasms. – Subject Index.

Reproductive Biology

J. Dietl, University of Tübingen (Ed.)

The Mammalian Egg Coat

Structure and Function

Foreword by P. M. Wassarman

1989. 66 figures. XVI, 156 pages. Hard cover DM 126,–. ISBN 3-540-50272-6

The book presents the most up-to-date information concerning the field of gamete interaction in reproductive biology. Especially interesting is the extensive account of the site of synthesis of the extracellular egg coat, which has been a matter of dispute for decades.

M. C. Chapman, J. G. Grudzinskas, T. Chard, London (Eds.)

Implantation

Biological and Clinical Aspects

1988. 74 figures. XII, 258 pages. Hard cover DM 180,–. ISBN 3-540-19533-5

Outlining current research in relation to the establishment of human pregnancy, the areas of endocrinology, protein biochemistry, prostaglandins and immunology are all covered.

Pathology

F. Nogales, University of Granada (Ed.)

Ovarian Pathology

1989. 62 figures, some in color. Approx. 180 pages. (Current Topics in Pathology, Volume 78). Hard cover DM 148,–.
ISBN 3-540-50217-3

Great emphasis in this up-to-date text is placed on problems arising from surgical-pathological staging of ovarian tumours, such as the integrative role of the oncologist-pathologist team. Two subjects which are seldom reviewed in the literature – hormonally related non-neoplastic conditions and the ovary in infertility – are given separate chapters.

H. Rotterdam, New York University Medical Center; **H. T. Enterline,** University of Pennsylvania

Pathology of the Stomach and Duodenum

1989. 220 figures. VII, 320 pages. Hard cover DM 248,–. ISBN 3-540-96823-7

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1988. 32 figures. VI, 231 pages. Hard cover DM 128,–. ISBN 3-540-19156-9

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Claims on tissue plasminogen activator

R. Stephen Crespi

A 'master' patent on a recombinant protein product has twice been overturned in British courts. The consequences may be far-reaching.

THE struggle between Genentech and the Wellcome Foundation over Genentech's British patent for tissue plasminogen activator is of keen interest world-wide. Tissue plasminogen activator (TPA) converts plasminogen into plasmin, the enzyme that breaks down fibrin clots formed in coronary thrombosis, and has a large potential market. Britain is the arena for the first patent litigation on this biotechnology product, but the issues at stake transcend national boundaries and the judgement of the British courts will have international influence.

The patent in suit was obtained through the British system, but Genentech also has a parallel patent application in the European Patent Office, which will probably be granted no later than mid-1989. Depending on the claims that issue, the European patent will give Genentech an apparent second chance to protect its British market if the British national patent, which was rejected in July 1987 in the British Patents Court and again by the Court of Appeal in October 1988, is finally held invalid on appeal to the House of Lords. At the time of writing Genentech has yet to appeal.

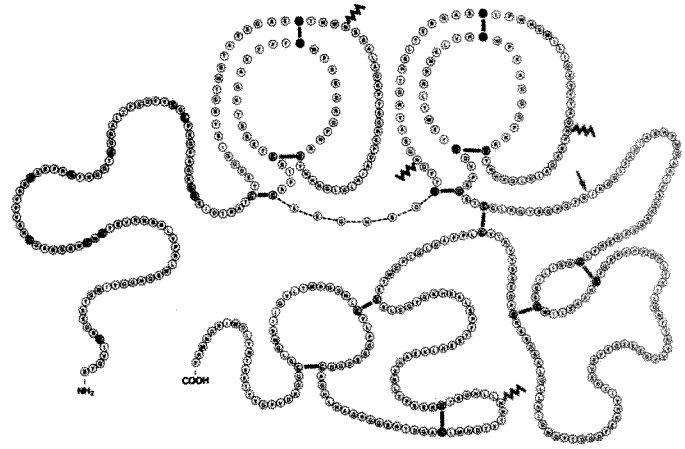
Genentech has a corresponding patent in the United States, which was granted in August 1988. This claims the DNA sequence that encodes TPA as well as expression vectors and transformed microorganisms containing this gene, but not the final TPA product. Litigation on the US patent has also begun.

The significance of the TPA case in Britain is that Genentech is strenuously pursuing patent claims for the recombinant protein product as such, and for correspondingly broad processes, rather than a claim for any detailed method limited to particular plasmids chosen to carry the TPA cDNA into the particular host cells used by Genentech to express the protein product. The case is therefore a prototype for other patents in the pipeline covering other blood proteins — including factor VIII, antithrombin and serum albumin — and may well be a pointer to the evaluation of the patent system in relation to similar biotechnology products. It is not surprising, in view of the decisions handed down so far, that some commentators have suggested devising another type of legal protection that would recognize with more certainty the achievements of those who are first to succeed in producing valuable proteins by genetic engineering.

To appreciate the issues in the TPA case, it is necessary to understand how patents are constructed. A patent specification consists of two parts: first, the technical description, which sets out the nature of the invention and instructs the skilled worker how to reproduce it; second, the legal definitions, known as the 'claims', which circumscribe the protection given by the patent. The claims are verbal formulae defining the protected technology in terms of process, product, use or some other category. Here the wording is important, although the courts have always allowed some elasticity of interpretation in order to catch clever evaders of the literal text. The European Patent Convention tries to strike a balance between the extremes of tight literal wording, on the one hand, and woolly statements of uncertain scope on the other.

A claim to the product itself (a *per se* claim) is possible when the product is new (that is, it has not been previously described). But where the product is not new *per se*, but only the process for making it is new, it is possible to claim the product as made by the particular process (a product-by-process claim). As human TPA was not a totally new substance at the priority date of Genentech's first patent application (in the United States), it was appropriate to use the more restricted product claim, as exemplified by claim 3 in the TPA patent, which reads: "Human tissue plasminogen activator as produced by recombinant DNA technology".

Patent disputes centre almost entirely upon the claims. The patentability conditions of novelty, inventiveness and industrial application or utility are applied solely to the claimed subject matter. The other main condition is that the inventor must provide a sufficient or 'enabling' disclosure. The disclosure requirement applies to the technical description; and if a claim is so broad as to cover subject matter for which the directions given to make the product or perform the process are seriously defective, the patent will be invalid.



Desirable form — diagram of the potential structure of human TPA, published by Pennica et al. *after the priority date of Genentech's first patent application (*Nature *301, 219; 1983).*

Here, I should dispose of a common misunderstanding. It is incorrect to assume that products of nature cannot be patented on the grounds that, by reason of their previous existence in nature, they cannot be novel. Patent law has become much more liberal on this point by taking into account the merit of first discovery of a natural substance, its isolation and provision of a product in a form useful for therapeutic or other purposes. The guidelines of the European Patent Office follow this approach and endorse the patentability of natural products in the proper circumstances. Of course, TPA had been isolated from human tissue before Genentech began work, and the identical product had also been produced by culture of the Bowes melanoma cell line. The TPA claim quoted above was therefore appropriate. As defined, the product is not one of nature but of a non-natural process.

Genentech based its case on the position stated in the patent itself:

The present invention arises in part from the discovery of the DNA sequence and deduced amino acid sequence of human plasminogen activator. This discovery enabled the production of human plasminogen activator via the application of recombinant DNA technology, in turn, enabling the production of sufficient quality and quantity of material to initiate and conduct animal and clinical testing as prerequisites to market approval, unimpeded by the restrictions necessarily inherent in the isolation methods hitherto employed involving production and extraction from existing cell culture. This invention is directed to these associated embodiments in all respects.

The Patents Court held that broad product claims such as claim 3 would only have been acceptable if the patentee had been the first to discover TPA or its desirable properties. Although Genentech had been the first to identify the DNA-coding sequence and to deduce the amino-acid sequence of the protein (in the court said to be "a remarkable job of work" which "involved laborious and costly effort") it had already been recognized by other workers as desirable to produce TPA in quantity by recombinant DNA methods. Genentech's product claims were therefore too wide in being directed to a possible and obviously desirable end that could be reached by routes on which only limited guidance had been given. Only a limited process claim could be upheld in these circumstances.

Last October, at least four crucial questions emerged in the Court of Appeal. What precisely did Genentech *invent* (rather than merely *discover*)? What scope of claim would properly reflect the invention? Was the claim to recombinant TPA obvious to the skilled worker at the relevant date? And what level of skill and knowledge would the hypothetical ordinary skilled worker be assumed to have in relation to the previous question?

Key contribution?

Genentech stuck to the assertion that the discovery of the sequence was the key contribution, the 'one log holding up the log jam' in the way of progress to the desired product. But both British and European law state that a discovery, as such, is not patentable, and it is universal law that you cannot patent knowledge itself but only its practical application. One judge floated the idea of subtracting the discovery element and seeing whether what was left amounted to an inventive step. This is a dangerous line to follow for any invention in the natural sciences, and fortunately the final judgement will not rest on this type of analysis.

On scope of claim, Genentech pursued a very bold line. The company argued that the discovery of the cDNA sequence led not only to the production of the recombinant form of natural TPA having the amino-acid sequence 1 to 527 shown in Fig. 5 of the patent drawings, but also opened the way to modifications of the natural molecule (for example, by site-directed mutagenesis of the underlying DNA) which preserved the essential TPA-type activity. This extension of the claim was of no great importance as regards Wellcome's intended product, but shows that Genentech was seeking to dominate a wide range of second- and subsequent-generation products in this field. To claim for the TPA patent the status of a master patent on the basis of discovering the sequence of TPA alone was remarkably daring. The Court of Appeal found this far-reaching proposal unacceptable and was not prepared to sustain claim 3 even if limited to the recombinant equivalent of the natural product. Thus, one judge pronounced: the "contention that Genentech should be protected against any use of this information howsoever this may be achieved in the future is . . . not a claim to a method embracing the discovery but rather a claim for protection of the discovery as such".

In patent disputes over new chemical compounds, it has been argued that something is obvious if a number of research groups are working along similar lines. This argument has not been well received. In the TPA case, however, it succeeded, as seen in the following extracts from the judgement:

It is agreed between the experts that at the relevant time, in early May 1982, the production of quantities of TPA adequate to treat human patients suffering from blood clots was known to be a desirable objective and it is further agreed that the Bowes melanoma cell-line was then available and was known to be a source of TPA and of the corresponding mRNA. It was of course then known that the nucleotide and amino-acid sequences of TPA existed, though their composition was not known. All the steps taken by Genentech in finding out the composition of the sequences and applying that knowledge to produce human TPA, as defined in the specification, by recombinant DNA technology were applications of known technology, and no step was by itself inventive. . . .

. . . it was indeed obvious, in my judgement, to the person skilled in the art to set out to produce human TPA by recombinant DNA technology. At least four teams did just that at about the same time. The evidence of those at Leuven and Umea [other research groups] as to their choice of projects is particularly relevant. The end was a known desideratum, and to proceed by oligonucleotide probing was, if not the first choice of each team, an early choice which lay in the way, ready to hand.

Wellcome thus persuaded two of the three judges in the Court of Appeal that the product claims were invalid not only because they were conceptually obvious but also because their practical realization involved no inventive step. Wellcome did not fare so well with the additional contention that the claims were also of unfairly wide scope in light of the technical description. This is a matter that the Patent Office can raise when it examines a patent application. But once a patent has been granted, the Court of Appeal held that it cannot be re-opened under the permitted grounds on which patents can be revoked. This conclusion stands in sharp contrast to the long-standing tradition in British patent law that the courts can also consider the question of fairness of the scope of a claim in the light of the patent description. If Wellcome counter-appeals against this point, the House of Lords must decide whether the 1977 Patents Act denies to the courts a remedy open to the lowest level of authority in the British patent system.

New problem

What then is unique or new about the TPA case? One Appeal judge said:

. . . we are here dealing with a new problem. New not only because the technology is very recent, but also because the problems arising from the manufacture of an existing substance with known properties, by the application of a known technology, through the medium of a new discovery and novel intermediate artifacts (the vectors) has never (so far as I can see) been considered by the courts.

The final answer, however, has been clear and unanimous from all judges in the case, even though their individual approaches vary distinctly. The same judge also said:

. . . the importance of the present action far outstrips the ultimate decision itself, for it is impossible to arrive at a decision without expressing conclusions which apply to this fledgling industry as a whole, and which (if reflected in courts elsewhere in the European Community and outside) may have an important bearing on its future.

However hard the judgements may seem, they simply result from the application of standard principles of patent law to the cloning of DNA that encodes naturally occurring proteins. For some time it has been an open question whether such cloning involves invention as distinct from experimental skill and the application of sufficient resources. But it is difficult to see how a different legal system would cope better with the situation. Copyright as an alternative to patent law is a blind alley in relation to DNA sequences, and no other new system is yet on any lawyer's drawing board. Moreover, the difficulties are not nearly as great for newly isolated natural proteins and the products of protein engineering; here the question of inventiveness will return to the more familiar field of new compound chemistry. Given that the complexities of the Genentech case stem from transitional problems, it is premature to despair of the patent system for the protection of innovation in biotechnology. □

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The physics of dense nuclear matter from supernovae to quark gluon plasma

Reinhard Stock

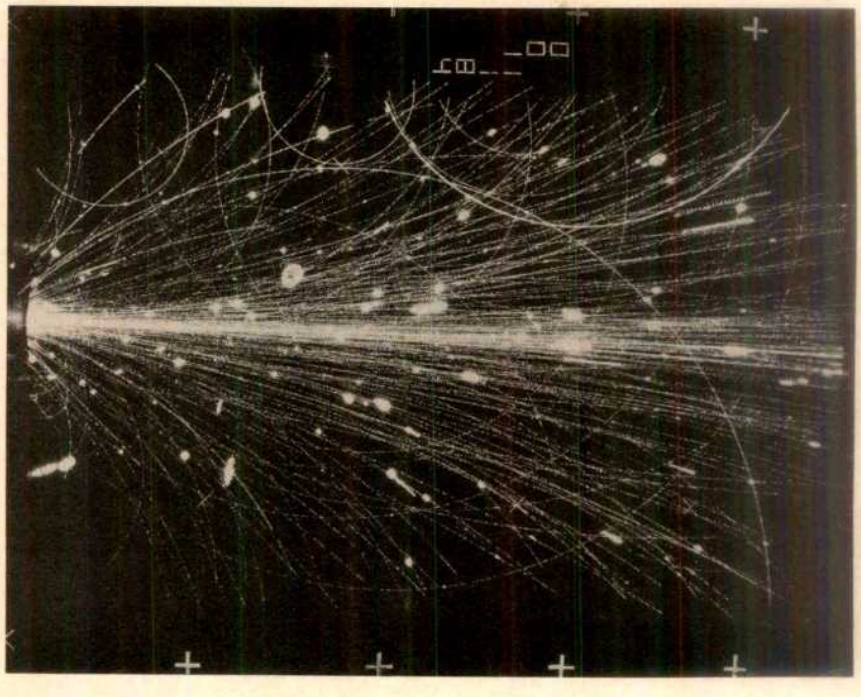
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At high density, nuclear matter is better regarded as a dense fluid rather than a collection of individual protons and neutrons. A good understanding of the properties of nuclear matter, important in the physics of neutron stars and the Big Bang as well as heavy atomic nuclei, is now emerging.

IN light atomic nuclei, the constituent protons and neutrons retain some of their individual identity and occupy distinct energy states, but in the interior of heavy nuclei, where the density is higher, the available energy states are closer together and more numerous, and the neutrons and protons form a continuous, fluid-like medium known as nuclear matter. Ground-state nuclear matter is stable because the powerful attractive and repulsive components of the strong interaction nearly cancel out. The additional electromagnetic interaction—Coulomb repulsion between the charged protons—causes a stability limit at nucleon number $A \approx 210$, close to that of lead and bismuth. Were it not for this interaction, nuclear matter could form stable volumes of any microscopic or macroscopic size. Stability returns only when the atomic number becomes very large, $A \gtrsim 10^{57}$. Then gravitational attraction is enough to balance the repulsion of the strong interaction, permitting the formation of neutron stars.

Despite decades of research in nuclear physics, we still do not understand nuclear matter very well. Until recently, the physicists' task was seen largely as the construction of detailed models for isolated nuclear species and their excited states, and for the interactions between them. Nuclear matter was regarded as an abstract extrapolation from studies of nuclear structure. This attitude changed about 20 years ago, when astrophysicists began to develop semi-quantitative models for supernova dynamics, neutron stars and the evolution of the Big Bang, all of which demanded detailed understanding of the continuum properties of extended nuclear matter or, more generally, of strongly interacting matter at high density. This need prompted the research described here.

The ground-state density in nuclei is 2.7×10^{14} g cm^{-3}, corresponding to a numerical nucleon density of $\rho_0 = 0.15$ fm^{-3} ($1\ \text{fm} = 10^{-15}$ m) and an energy density $\varepsilon_0 = 0.14$ GeV fm^{-3}. Familiar nuclear reactions such as fission, fusion and alpha-decay proceed with only small changes in density, of a few per cent: nuclear matter flows gently between its various 'droplet' species. Type II supernovae (that is, those occurring among young, massive (Population I) stars) may, on the other hand, reach densities of about $4\rho_0$, and still higher densities are proposed for the interior of heavy neutron stars. To understand supernova dynamics, the stability and static architecture of neutron stars, and the critical limits governing black-hole formation, we need to know the macroscopic properties of nuclear matter, such as compressibility, specific heat and entropy density. In short, we need to know the equation of state of nuclear matter (the relationship between pressure, density and



A streamer-chamber photograph of a central collision between ^{16}O and Pb at the CERN SPS, with an energy of 200 GeV per nucleon. It illustrates the high multiplicity of final-state events which must be scrutinized for evidence of the transient formation of quark matter during the collision. The magnetic field in the detector separates positive and negative particles, bending them according to their momenta, and track brilliance measurements distinguish particle types. The tracks in the pencil surrounding the beam axis,corresponding to the forward part of the phase space, cannot be resolved, but are of minor significance in the analysis.

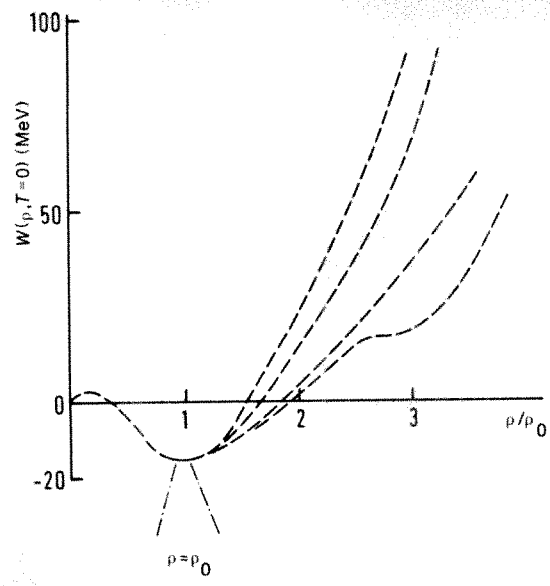


Fig. 1 Sketch of the energy-density function (the energy per particle) of nuclear matter. The internal energy per nucleon of cold ($T = 0$) matter has a well-known minimum at density $\rho = \rho_0$, corresponding to the matter inside stable nuclei. Towards higher densities the energy should increase.

temperature) over a wide range of density, far beyond that of the nuclear interior.

History

The work described in this article had the goal of achieving high compression of nuclear matter in the laboratory by colliding heavy nuclear projectiles 'head-on' at relativistic energy. The concept of shock compression in such heavy-ion collisions was analysed theoretically between 1973 and 1975 by H.A. Bethe at Cornell, G. Chapline at Lawrence Livermore Laboratory, W. Greiner at Frankfurt and their collaborators[1-3]. At the same time, experimental investigations were made possible by construction of the Bevalac accelerator at Lawrence Berkeley Laboratory (LBL). By combining a low-energy heavy-ion linear accelerator with the old high-energy proton synchrotron (Bevatron) the Bevalac first provided beams of nuclei up to $A = 40$ (calcium) at energies up to 2 GeV per projectile nucleon. With a total kinetic energy about twice the rest-mass of the nucleus, the projectiles move at relativistic velocity ($v \approx 0.9c$). Technical improvements circumvented the limitation to $A \leq 40$, permitting beams of gold and uranium nuclei to be produced. Similar work at Dubna, Soviet Union, reached higher energy (3.5 GeV per nucleon) but only for nuclei up to $A = 20$ (neon), adding an element of competition to the emerging field of relativistic heavy-ion physics.

At Berkeley, the search for the nuclear equation of state was carried out, in particular, by three major international collaborations of physicists with backgrounds in both nuclear and particle physics. A collaboration between Japan and the United States focused on the development of magnetic spectrometers, and two German-US groups constructed complementary types of 4π-detector systems, the visual-track-recording streamer chamber[4] and the electronic Plastic Ball detector[5], which simultaneously measure all particles created in a collision event. By the early 1980s, the German-US collaboration had succeeded in an initial demonstration of the shock-compression mechanism and arrived at a first semi-empirical estimate of the equation of state in the density domain $1.5 < \rho/\rho_0 < 3.5$ (refs 4, 5). But a different conclusion was reached at about the same time from the dynamical model for the so-called prompt supernova-bounce mechanism[6], which indicated an ultra-soft (very low compression energy) equation of state. This was in strong disagreement with the very stiff (high compression energy) equation of state indicated by the results from heavy-ion collisions. Subsequent theoretical work has arrived at a possible reconciliation of the two results. A new generation of refined experimental inverstigations, both at LBL and with the synchrotron-storage ring combination which is now under construction at GSI Darmstadt, will be based on these ideas.

Experiments in the GeV-per-nucleon energy domain move far away from familiar ground-state nuclear matter but do not reach the very limit of dense matter consisting of hadrons (nucleons, baryon resonances, pions, kaons and so on). The gauge theory of the strong interaction, quantum chromodynamics (QCD), leads one to expect a phase transition at a high hadronic packing density, in which the quarks and gluons escape from of their confinement within hadron 'bags' and move instead through an extended quark-gluon medium termed quark matter[7]. A modern statistical method used to obtain the QCD equation of state, lattice-gauge QCD, has predicted that this phase transition from hadronic to quark matter should occur at energy densities of $\varepsilon \approx 2$-3 GeV fm^{-3}, about 20 times the ground-state nuclear-matter energy density[8]. The Bevalac and future similar accelerators do not reach beyond ~ 0.7 GeV fm^{-3}.

The quark-gluon plasma is a primordial medium whose properties are dictated by the strong force between quarks. It probably existed during the first microsecond (the quark era) of the Big Bang, just before the 'freezeout' into recognizable hadrons. Demonstration of its existence and determination of its critical density, temperature and transition enthalpy would not only provide a novel check for QCD but would also aid our understanding of the composition and distribution of matter in the hadronic period of the Universe, which still exists today. Far higher accelerator energies are required to probe this state. A programme of heavy-ion reaction studies was thus begun at the Alternating Gradient Synchrotron (AGS) at Brookhaven National Laboratory, and at the Super Proton Synchrotron (SPS) at CERN, Geneva. Both have beams of $A \approx 30$ (^{28}Si, ^{32}S), the AGS reaching 14.5 GeV per nucleon, the SPS up to 200 GeV per nucleon. In first runs during 1986-87, new international experimental collaborations have employed vastly more complicated and diverse instruments than earlier studies at the Bevalac and in Dubna. The first results seem to indicate that energy densities of 2-3 GeV fm^{-3} are indeed attained and some initial observations are briefly described at the end of this article.

The nuclear equation of state

The mechanics of continuous media are described in the equation of state, which specifies the pressure as a function of density and temperature, $P(\rho, T)$. For microscopic models, the energy per particle, $W(\rho, T)$ is a more convenient quantity. Knowledge of W is, in principle, equivalent to knowing the equation of state, because

$$P(\rho, T) = \rho^2 \frac{\partial W(\rho, T)}{\partial \rho} \quad \text{(at constant entropy)} \qquad (1$$

and the term equation of state is often loosely employed for the function $W(\rho, T)$. In the absence of thermal excitation, that is for $T = 0$ states such as nuclear ground states or neutron-sta

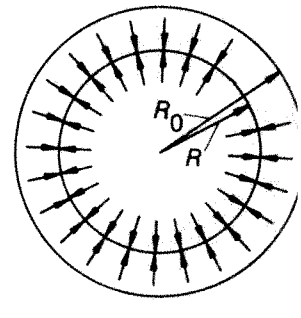


Fig. 2 Sketch of hydrostatic equilibrium in a neutron star of radius R_0. At each subshell with radius R the compressional nuclear matter pressure has to counterbalance the gravitational inward pressure of the layer from R to R_0.

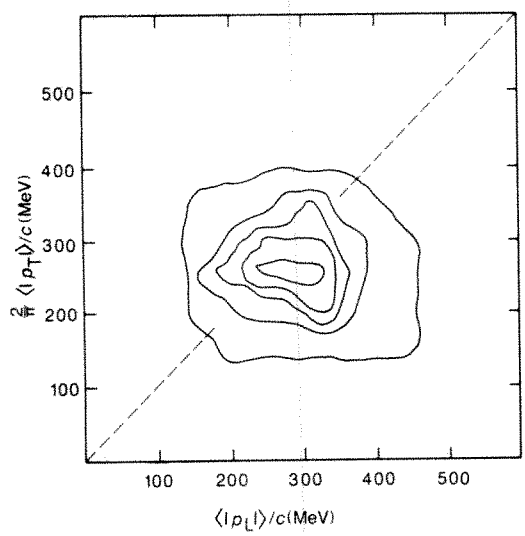


Fig. 3 The achievement of a temporary thermal equilibrium in nucleus-nucleus collisions requires that the momentum of the resultant body in the mean transverse ($\langle|p_T|\rangle$) and longitudinal ($\langle|p_L|\rangle$) directions are equal. The data for collisions of ^{40}Ar and Pb at 0.8 GeV per nucleon demonstrate that this is fulfilled on average: the contour lines of event frequency (each spaced by a factor of two) show a 'mountain' at the diagonal.

matter, it is sufficient to know the compressional energy $W(\rho, T=0)$, from which the $T=0$ equation of state follows:

$$P(\rho, T=0) = \rho^2 \frac{\mathrm{d}W(\rho, T=0)}{d\rho} \qquad (2)$$

Figure 1 illustrates the qualitative expectations for $W(\rho, T=0)$ of nuclear matter. The minimum at $\rho = \rho_0$ represents the stability of ground-state matter: $P(\rho_0, T=0) = 0$ from equation (2). $W(\rho_0, T=0)$ is the familiar mean binding energy per nucleon, W_0, given by the Bethe-Weizsäcker formula. The assumption of a linear response to perturbations about $\rho = \rho_0$ yields

$$W(\rho, T=0) = \frac{K}{18\rho_0^2}(\rho - \rho_0)^2 + W_0 \qquad (3)$$

$P(\rho)$ then crosses the $P=0$ axis with slope $(\mathrm{d}P/\mathrm{d}\rho)_{P=0} = K/9$, and the stiffness of the medium is given by the compressibility K, which also fixes the sound velocity. An estimate of K can be obtained from the excitation energy of the nuclear monopole vibrational state, which has been successfully described as a collective density oscillation about ρ_0. The canonical semi-empirical value thus derived was previously considered to be $K = 220 \pm 30$ MeV (ref. 9) but new data and theory now indicate $K = 300 \pm 25$ MeV (ref. 10), a compression response which must be considered 'stiff'; a 'soft' response corresponds to $K \approx 150$ MeV.

So what is there left to do? Figure 1 shows that there are vastly different possible forms for W at $\rho > 1.5\rho_0$. The monopole vibration covers only a tiny density interval, $\rho = \rho_0 \pm 2\%$, in which it confirms the parabolic shape (equation 3) and yields a value for the constant K. W cannot remain parabolic at high ρ/ρ_0 because nuclear matter would behave acausally (sound speed $v_s > c$) even at neutron-star densities. The parameter K alone does not describe $W(\rho, T=0)$ and may be totally irrelevant for $\rho > 2\rho_0$. In this domain W should of course grow with ρ because the short-range nuclear interaction is repulsive. This can be understood qualitatively by observing that the average distance r of neighbouring nucleons is about 1.8 fm in ground-state, $\rho = \rho_0$ matter, which is just twice the nucleon 'radius' $R = 0.9$ fm found from electron scattering[11]. At $\rho = \rho_0$, the nucleons are thus already tightly packed, and at fourfold compression they would get squeezed to $r \approx 1.1$ fm, so that their internal quark wavefunctions would overlap significantly. The Pauli principle would then force some of the quarks up to orbits of higher energy and this, crudely speaking, is the energy expended during compression. W thus grows with ρ—but how steeply?

Consider neutron stars, for example. Figure 2 illustrates the relevance of the equation of state for radial density distribution and stability. Hydrostatic equilibrium demands that the inward gravitational pressure is compensated by an equally large outward pressure from nuclear-matter incompressiblity, at each radial shell of thickness $R_0 - R$ (where R_0 is the radius of the star). $P(\rho, T=0)$ thus determines the stellar density distribution $\rho(R)$. If the total mass is too high or the equation of state is too soft at high ρ, there is no stable solution and gravitation creates a black hole. If the strong interaction could be removed, the Fermi energy of the degenerate neutron gas alone would let $W(\rho, T=0)$ increase as $\rho^{2/3}$, and the maximum mass would be the well-known Chandrasekhar limit of 1.4 solar masses ($M_\odot$) (refs 12, 13). But neutron stars of up to 2 $M_\odot$ are known[14], so the strong interaction must add to the repulsion. For $\rho \gtrsim 2\rho_0$, $W(\rho)$ thus grows faster than $\rho^{2/3}$ but slower than ρ^2.

Shock compression in relativistic collisions

Shock compression occurs when portions of a medium move through each other at supersonic velocities. If two equal-mass heavy nuclei collide head on at a beam energy of 1 GeV per nucleon, the interpenetration velocity of the target and projectile nuclear matter in the centre-of-mass frame is about $0.7c$, well above the velocity of sound or of any other mechanism that could transport energy or matter out of the reaction volume. Matter and energy therefore pile up in the shock zone, producing the desired compression in a 'fireball' volume. The hydrodynamical model of shock formation[1-3] predicts a fireball density of $\rho \approx 3\rho_0$ at this energy, a temperature[15] of about 80 MeV ($\sim 10^{12}$ K) and a lifetime of $\sim 5 \times 10^{-23}$ s for heavy colliding nuclei ($A \approx 200$). Afterwards, the compressed zone expands, again passing through $\rho = \rho_0$ and exploding into non-interacting hadrons at $\rho < 0.3\rho_0$. The overall dynamical cycle is a sequence of shock compression, fireball, expansion and freezing out.

It took some time before suitable experimental probes for the high-density phase were devised. Before describing these, it is worth considering why the thermodynamic model should be applicable to these reactions. In particular, this model assumes that the shock volume (the fireball) is at rest in the centre-of-mass frame. The two interpenetrating volumes of nuclear matter thus have to stop down their initial relative motion, producing a fireball in which the transverse and longitudinal motions are equally distributed. This nuclear stopping process arises from elastic and, more effectively, inelastic scattering of the incoming nucleons, which populate the transverse degrees of freedom in the fireball, as well as pion production, isobar excitation and compressional (potential) energy. Essentially all the terms in the energy-density function $W(\rho, T)$ are separate agents creating internal centre-of-mass energy out of initial longitudinal energy. The degree of stopping is easy to check. Figure 3 shows streamer-chamber data[16] for head-on collisions between ^{40}Ar and Pb at 0.8 GeV per nucleon. For each collision, the mean transverse ($\langle p_T \rangle$) and longitudinal ($\langle p_L \rangle$) momentum of all final-state particles was determined, giving one point in the $\langle p_T \rangle$–$\langle p_L \rangle$ plane. Equipartition requires $\langle|p_T|\rangle = \pi/2\langle|p_L|\rangle$ in the centre-of-mass frame, and the data show that this is fulfilled on average. From the same experiment the total transverse energy is found to be in good agreement with the kinematic prediction under the assumption of complete stopping.

One of the key questions in extracting the W function from the data is that of how the internal energy is shared between thermal motion, compressional energy, newly created rest mass and so on. If we combine the rest-mass (m_0) component with the thermal energy (because the new $m_0c^2 \to 0$ as $T \to 0$), we could expect to determine the compressional energy

$W^{comp}(\rho, T=0)$ from the known total centre-of-mass (c.m.) energy (assuming full stopping) by an appropriate measurement of the thermal energy fraction W^{therm}:

$$E \text{ per nucleon (lab)} \rightarrow E \text{ per nucleon (c.m.)} \approx W^{comp}(\rho, T=0) + W^{therm}(\rho, T). \quad (4)$$

In other words, the ground-state function $W(\rho, \tau=0)$ can be approximately found if the thermal component can be measured and subtracted. The idea that the pion yield closely reflects the thermal energy fraction (intuitively, pions are created out of collision energy, with the potential energy remaining passive)[2,17] was exploited by the GSI-LBL streamer chamber collaboration[18,19]. Figure 4 illustrates how the supposed compressional energy was determined from systematic measurement[20] of the pion yield in central collisions of equal-mass pairs at $A \approx 40$ (Ar and KCl) and 140 (La and La). The ratio of pions produced to nucleons participating, $\langle n_\pi \rangle / A$, increases linearly with the centre-of-mass energy per nucleon above ~100 MeV per nucleon.

A calculation employing a thermochemical pion-production model that contains no potential energy gives a pion yield that increases more steeply with energy than does the experimental data. An arbitrary pion concentration is thus reached at an energy ε', according to the thermal model, which is lower than the energy ε when that concentration is actually observed. The difference between ε and ε' was interpreted as the potential energy expended in reaching the density $\rho(\varepsilon)$ from initial ground-state density ρ_0. This simple-minded assumption gives $\varepsilon - \varepsilon' \approx W^{comp}$ as a function of the centre-of-mass energy ε. From the Hugoniot-Rankine equation of hydrodynamic shock compression[1-3], ρ was determined as a function of ε, finally yielding a semi-empirical estimate of $W(\rho, T=0)$. The results[20] and a critique of this procedure are discussed below.

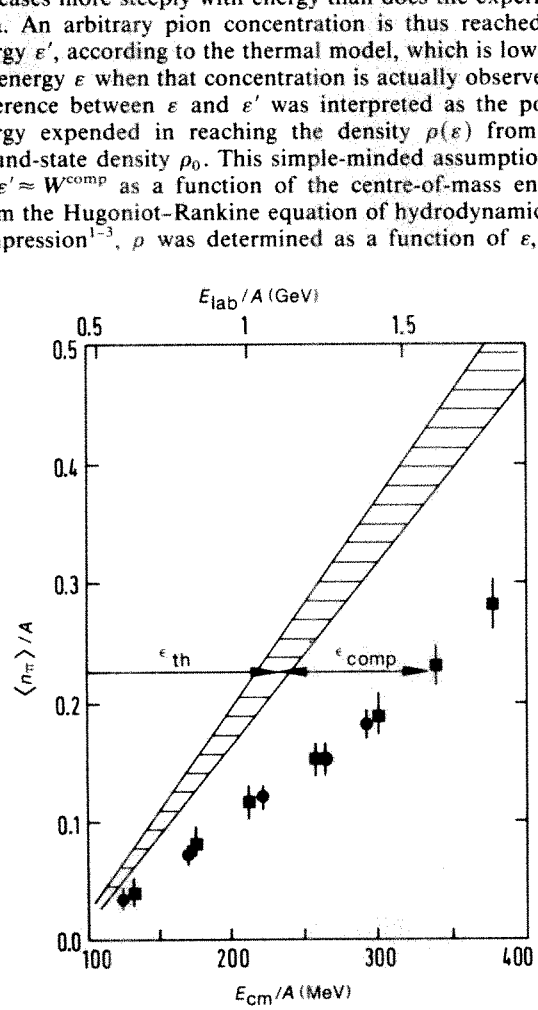


Fig. 4 Pion production rates from the fireball produced in a nuclear collision should be proportional to the thermal energy ε_{th} regardless of the potential energy. Data for the average number of pions produced $\langle n_\pi \rangle$ per nucleon participating in central collisions of Ar and KCl (squares), and La and La, (circles), as a function of the energy per nucleon in the centre-of-mass (E_{cm}, bottom scale) and laboratory (E_{lab}, top scale) frame, are used to estimate the compressional (potential) energy ε_{comp} according to equation (4). The hatched region indicates the predictions of thermal models. In these experiments, solid KCl was used as a target as the nearest non-gaseous, non-conducting equivalent to the Ar-atom projectiles (of mass 38).

Instead of trying to determine the energy function $W(\rho, T=0)$ why not aim directly at the pressure $P(\rho)$? The long-standing prediction[21] that collective pressure would lead to a sideways flow of emitted baryons was finally verified by the Plastic Ball collaboration[22]. Just as a water droplet splashes sideways on impact, nuclear matter develops a pressure field during interpenetration that displaces matter perpendicular to the beam axis. The final-state momentum distribution of all participating nucleons should thus be cigar-shaped in momentum space, a triaxial ellipsoid with its longest axis in the direction of favoured baryon flow. Its angle with respect to the beam axis is called the flow angle. When other variables are kept constant, the flow angle increases with the pressure in the reaction zone and so with the stiffness of the equation of state.

Figure 5 shows the results[23] from the Plastic Ball experiment for the flow angle in collisions between two gold atoms at various energies. This spectrometer has 971 single detectors arranged in a tightly-packed ball to measure particle emission angle, energy and identity for all charged final-state products, and has a planar surface to pick up the small forward angles [5]. The recorded events were first grouped according to their charged-particle or proton multiplicity (number of charged particles or protons created), where a low multiplicity is known to correspond to a peripheral collision and a high one to a central collision. The momentum tensor was then diagonalized for each event, yielding the flow angle Θ. The histograms in figure 5 show the distribution of $\cos\Theta$ for various bins of multiplicity and at five incident energies. In low-multiplicity events, the distribution peaks at $\Theta = 0°$: no preferential sideways flux occurs in grazing collisions, where only the dilute nuclear surfaces interact and there is little compression. A clear sideways peak developed for high-multiplicity events (near-central collisions).

Information about the equation of state can be obtained by comparing the data to theoretical calculations of the reaction dynamics that use a parameterized equation of state. This use of macroscopic models, such as hydrodynamics, and microscopic models that follow the nucleon collision cascades makes it clear that the equation of state is not directly measurable. All results from heavy-ion collisions require auxiliary models and are thus semi-empirical.

Nuclear collisions and astrophysics

The outcome of heavy-ion collision work on the nuclear-matter energy-density function $W(\rho, T=0)$ as it stood in 1986 is shown in Fig. 6, which includes results from the streamer chamber investigation of the 'pion thermometer'[18-20] as well as transverse energy data[24] measured by the Plastic Ball collaboration using a variation of the assumptions embodied in equation (4). These points agree closely with the W function deduced from fitting the flow angle and related data. The microscopic models employed here[25-27] were developed mostly by Stöcker, Aichelin and Bertsch as an extension of simple models of collision cascades by including the Pauli principle, microscopic effective nucleon-nucleon interactions and an average mean field, giving the nucleons a potential energy. This type of theory is known as the Vlasov-Uehling-Uhlenbeck (VUU) model. Figure 6 indicates a stiff equation of state. Note, however, that the data points start at a density $\rho/\rho_0 = 1.7$ and give no new insight into the result obtained from the monopole vibration in the immediate vicinity of $\rho = \rho_0$. But, as expected, W is approximately linear in ρ/ρ_0.

These results can be compared with astrophysical estimates of the equation of state for heavy neutron stars[28], also shown in Fig. 6. Analysis of 4UO900-40, a star with mass ~1.85 $M_\odot$, gives a much shallower W function. An even softer equation of state has been used by Baron, Cooperstein and Kahana (BCK) in the prompt supernova-bounce model[29]. The BCK model posits that a type II supernova arises from a compression-shock

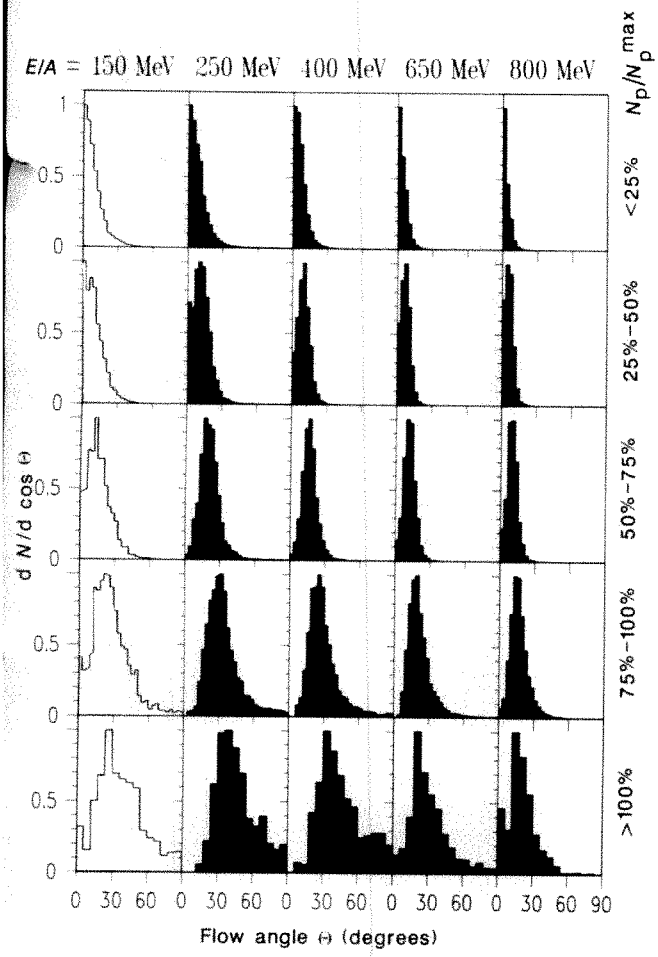


Fig. 5 In nuclear collisions at higher energies, the resultant blob of hot material is increasingly non-spherical. As a measure of this effect, the flow angle Θ between the beam axis and the longest axis of the fireball is measured. The figure plots the distribution of Θ in collisions of two gold atoms at five incident energies. The data are subdivided by multiplicity, the number of protons N_p in each event; five multiplicity bins are shown, with N_p divided by N_p^{max}, the average proton multiplicity in head-on events. N_p can occasionally exceed N_p^{max}. The flow angle peaks at 0° for low N_p (peripheral collisions) but develops a sideways shift at high N_p (central collisions).

explosion cycle of the iron core of a burned-out star of about 12–15 $M_\odot$, where the core has a mass of ~1.4 $M_\odot$. The shock explosion has to carry enough energy and matter outward to avoid being stalled by the mass of outer-shell material still falling inward. To create such a massive shock wave the collapse has to continue to very high density (small core radius) and thus traps a large amount of gravitational energy in the shock front that develops at the bounce point. The outer layer of the core then rebounds, the inner sections settling into a residual neutron star. A soft equation of state is required to reach high bounce-point densities, of $\rho \approx 4\rho_0$. The range of W functions that lead to a successful prompt-bounce simulation[29] is indicated in Fig. 6.

The first semi-empirical estimates of the energy–density function seem to be as contradictory as the qualitative *a priori* sketches (Fig. 2), but critical consideration of the theoretical models used in the derivation may remove some of the apparent contradictions.

● Nuclear collisions create hot hadronic matter, at temperatures up to 100 MeV, and contain up to 25% of their energy in pions and nucleon resonances. Supernovae, by contrast, are cold, proceeding at temperatures of about 10–15 MeV, neutron stars are at $T = 0$, and both are more nearly pure nucleon matter. The fireball nucleons move with a velocity of about $0.5c$ and the nucleon–nucleon potential is strongly velocity-dependent[30,31], so nuclear collisions do not reflect at all the

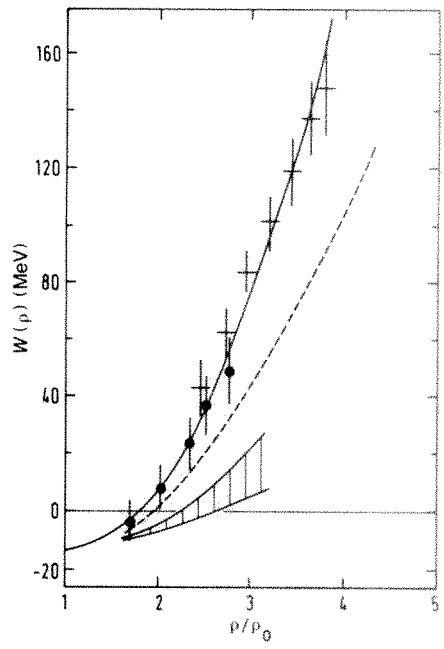


Fig. 6 The energy per nucleon, $W(\rho)$, can be obtained in a semi-empirical way by interpreting various observations with the help of simple theories. The figure shows the first generation of such estimates for $W(\rho)$, with ρ plotted against ρ_0, the ground-state nuclear-matter density. Heavy-ion collisions indicate a stiff response to compression (data points from pion data[20] (crosses) and radial energies[24] (circles), and VUU (solid) curve[25–27]) but the BCK model of supernova dynamics (hatched curve) demands a soft equation of state and indications from heavy neutron stars[28] (dashed curve) fall in between. These apparent contradictions may be due both to failings in the reaction models and to real differences between the dynamical conditions, such as temperature, in the different cases.

'cold' compressional energy. Our simplification (4) needs modification. Even with a correct model for the thermal energy $W^{therm}(\rho, T)$, the remaining fraction of the internal energy is not $W^{comp}(\rho, T=0)$ but a temperature-(velocity)-dependent $W^{comp}(\rho, T)$. First estimates of this effect indicate a considerable dynamical stiffening of the W function[31]. At $T = 100$ MeV and $\rho = 4\rho_0$, $W^{comp}(\rho, T)$ might exceed $W^{comp}(\rho, T=0)$ by 50 MeV. Thus the neutron star curve in Fig. 6 more probably represents the true $W^{comp}(\rho, T=0)$ and there are good reasons why the heavy-ion results are well above it.

● Theories have treated thermal degrees of freedom only to first order, ignoring possible shifts of nucleon, pion and resonance masses, and interaction cross-sections inside dense hadronic matter[32–34]. These 'effective masses' could increase the thermal part of the pressure, so that less static compressional pressure would be required to reproduce the sideways flow (Fig. 5). This might lower the VUU curve of Fig. 6 considerably.

● The prompt supernova-bounce model is by no means unchallenged[35–37]. There are many essential parameters in the physics of the simulation besides the equation of state that are not known with certainty, such as iron core mass, core entropy and degree of neutronization (the neutron concentration in the dense core). An alternative model[35,36] explains the supernova as a two-step process: the initial shock wave is allowed to stall at about 100 km radius but it is released again by reheating caused by absorption of a neutrino shower emitted by the neutronizing core. In this model stiffer equations of state than in the BCK model are admissible, but it remains to be shown that the energy release of the indirect mechanism suffices to account for the most recent supernova SN1987A.

The confrontration of data from nuclear experiments and astrophysics will obviously prompt further theoretical and experimental work, and the apparent contradictions may in the long run help to solve the puzzle of high-density hadronic matter.

Results from GSI's new heavy-ion synchrotrons will extend the work of LBL's Bevalac, but another nearby supernova would also be useful.

Outlook for quark matter

Whatever refinements and corrections to the equation-of-state data might be made in future, the smooth monotonic increase of $W(\rho)$ is unlikely to disappear. There is no indication yet of a phase transition, for up to about four times the ground-state density or for temperatures up to 100 MeV, where the total energy density amounts to ~ 0.7 GeV fm^{-3}. Lattice-gauge QCD, which suggests that hadronic matter is stable up to 2–3 GeV fm^{-3}, was first developed to study the quark–gluon wavefunctions of isolated hadrons, which have an energy density of ~ 0.5–1.0 GeV fm^{-3}, and was later adapted to study large volumes and high temperatures[8,38,39]. What accelerator energy is needed to match the critical conditions for the deconfinement transition from hadronic to quark–gluon matter? The step from 0.7 to 2.5 GeV fm^{-3} at first seems to demand a factor of about four in the centre-of-mass kinetic energy over and above the 0.4 GeV per nucleon attainable at the Bevalac, but at much higher energies we may not be able to count on the nuclear stopping power to accomplish complete thermalization, as observed in the Bevalac (Fig. 3). For example, at the energy of 32 GeV per nucleon obtained for the CERN Intersecting Storage Rings, a mere 15% of available centre-of-mass energy is thermalized in proton–proton collisions. Nucleus–nucleus collisions should provide greater stopping power because of sequential interactions in the extended medium, but we expect to need a twentyfold rather than a fourfold increase in the centre-of-mass energy, that is, 8–10 GeV per nucleon.

Because the CERN SPS could yield nuclear projectiles at 200 GeV per nucleon laboratory energy, which corresponds to 9.6 GeV per nucleon in the centre of mass, it was equipped with an injector for heavy ions by a collaboration between GSI, LBL and CERN. Using ^{16}O and ^{32}S projectiles, a set of pilot experiments were run to explore the new territory. The total beam energies of 3.2 TeV for ^{16}O and 6.4 TeV for ^{32}S set the world record for accelerator beams. The detectors are swamped with particles created in high-multiplicity events, as shown for a central collision of ^{16}O and Pb at 200 GeV per nucleon beam energy[40] (see panel on page 319). This event was recorded in the streamer chamber. About 280 charged particles are created in the collision, chiefly pions, kaons and protons. With an average spatial resolution of 1.5 mm, the total chamber volume of $2 \times 1.2 \times 0.7$ m^3 represents a potential information content of about 5 megabytes, enough to deal with multiplicities up to 500, such as those created in central interactions of ^{32}S and Au.

At CERN, a variety of techniques beside the streamer chamber are used to deal with this wealth of information: calorimetry to measure average energy-flow distributions, spectrometers for recording a small subfraction of the created particles at high precision, and some detectors that are set up specifically to find muon pairs or photons. Similar experiments have been conducted at the Brookhaven AGS synchrotron, with ^{16}O and ^{28}Si beams of 14.5 GeV per nucleon. Recent discussion of the first results from these new experiments indicates that the essential criteria for the formation of the quark–gluon matter are being met. Estimates[41] of the energy density reached in central collisions of ^{16}O and ^{32}S projectiles with heavy target nuclei are ~ 2.5–3 GeV fm^{-3}. This meets the critical deconfinement conditions predicted by lattice QCD, and shows that the nuclear stopping power is still effective at 200 GeV per nucleon. Pion correlation data demonstrate that a large, thermalized fireball volume with long lifetime is created at the centre of mass of the collision[42]. Furthermore, there is a suppression of the J/Ψ charm-meson production in central ^{16}O and ^{32}S collisions with U nuclei[43], which agrees with the prediction for charmonium production from deconfined matter[44].

Other data, on transverse momentum spectra, strange-particle production, photon production and total multiplicity are currently being examined for indications of new physics. Can they be understood by a simple reaction mechanism, such as cascading of standard nucleon–nucleon collisions, or do they require a different production mechanism which might bear the fingerprints of quark-matter production in the transient, high-density stage? A direct and unambiguous signature of quark-matter formation is still an evasive goal that awaits more theoretical work.

The study of GeV-per-nucleon-energy heavy-ion collisions has brought within our reach the equation of state for nuclear matter at densities relevant to supernovae and neutron stars. It has also added a new challenge to nuclear many-body theory by introducing relativity and a search for new dynamical models. Looking ahead, to increases in energy by factors of a hundred, the search for collective quark deconfinement is taking shape with discussions of suitable physics probes and instrumental development. The origin and nature of confinement is still an uncertain part of QCD, well proven only at short ranges (~ 0.1 fm), whereas confinement and deconfinement are intermediate-distance (0.5 fm) phenomena. Beside its fundamental interest, the existence and properties of a quark–gluon plasma state would be helpful in understanding of Big Bang expansion.

Until now, the two new research fields have not required a dedicated accelerator or large-scale experimental construction. GSI at Darmstadt is, however, now building a set of second-generation accelerators dedicated to nuclear-matter research. And experimenters at both CERN and Brookhaven are discussing the prospects for heavier projectiles, up to lead. Higher energies might follow later from the Large Hadron Collider project at Geneva, or the proposed Relativistic Heavy Ion Collider at Brookhaven. The analysis of the new data obtained with ^{32}S and ^{28}Si beams at CERN and Brookhaven will soon provide a firmer justification and direction for such developments.

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Reader Service No.16

Geophysical evidence for 'thick-skinned' crustal deformation in central Australia

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Until now, there has been no clear evidence from deep seismic reflection profiles of crustal faults that displace the Moho. New data from central Australia show such a structure in a region characterized by large thrust structures, large gravity anomalies and abrupt changes in teleseismic travel times. Here the principal thrust, the Redbank Deformed Zone, cuts through the crust, and the Moho is significantly displaced across it. The geological and geophysical evidence leads to a model of thick-skinned crustal deformation which occurred in an intra-cratonic environment in late Devonian time.

A CURRENT controversy in the study of continental tectonics is whether deformation initially involves the entire crust and the upper mantle (that is, a 'thick skin') or whether it is controlled by detachment zones within the crust so that much of the folding and imbricate thrusting is restricted to the upper crust alone (a 'thin skin')[1]. This controversy has been introduced recently to attempts to understand the formation of the Pyrenees of Europe, for which both thick- and thin-skinned interpretations have been proposed[2]. Well-known examples of thin-skinned tectonics include the Appalachians and Rocky Mountains of the United States and the Alps of Europe. Thick-skinned models are characterized by major planar crustal faults which cut the entire crust at moderately steep angles. Examples of thick-skinned tectonics include the Wind River Thrust of North America, the Grenville Front region of Canada and the Flannan and Outer Isles Thrusts of Great Britain. The Grenville Front in particular is an excellent example of thick-skinned tectonics[3]. Another example occurs in central Australia, and exhibits clear evidence of a major offset in Moho depth across the structure.

The tectonic setting of central Australia is characterized by a series of late Proterozoic–Palaeozoic basins and an exposed geological basement of middle Proterozoic age[4–7]. The basins include the Amadeus and Ngalia Basins, and the principal region of exposed basement is the Arunta Block (Fig. 1). The region as a whole is characterized by relatively flat topography and by major east–west-trending gravity anomalies (of peak-to-trough amplitudes exceeding 1,400 $\mu m\ s^{-1}$ (140 mgal)) (Fig. 1). Teleseismic travel-time anomalies of up to 1.5 s have also been recorded across the region over distances of a few tens of kilometres[8]. Major east–west-trending thrust zones occur within the Arunta Block. These observations, together with the geological evidence that the thrusts were last reactivated in an intracratonic environment in late Devonian to early Carboniferous times[9] with little subsequent tectonism, makes this basin-and-block structure quite unusual and raises a number of fundamental questions. What is the deep crustal structure beneath these basins and blocks? What were the driving forces that shaped these and other structures? How has this anomalous structure been supported since the time of the last major thrusting event[10]? Several models have been proposed in response to such questions[9–12], and a 500-km-long north–south deep-seismic-reflection traverse has been recorded by the Bureau of Mineral Resources across the Amadeus Basin and Arunta Block[13]. Only the northern section, extending from north of the Ngalia Basin to a point south of the major thrust zone (the Redbank Thrust or Redbank Deformed Zone), is discussed here (the section A–A′ in Fig. 1). Explosive sources were used throughout and the resulting deep-seismic-reflection data provide excellent resolution of the crustal structure down to an average depth of ~40 km.

Regional geology

The Amadeus Basin[4], bounded to the north by the Arunta Block and to the south by the Musgrave Block, contains up to 14 km of sediments of late Proterozoic (~900 Myr) to late Palaeozoic (~300 Myr) age. The maximum thickness of sediments occurs at the faulted and upturned northern margin, where the Arunta Block has been thrust up and over the basin sediments to form the Amadeus Homocline. Several deformation events have been identified within the basin sediments, with the major and final one having occurred at the time of the Alice Springs orogeny in the late Devonian to early Carboniferous. The northern margin of the Ngalia Basin is also fault-controlled and the last major orogenic event (the Mt Eclipse orogeny) occurred at about the same time as the Alice Springs orogeny[5]. The Ngalia Basin is not an important feature in the seismic-reflection section discussed here because the sediment thickness does not exceed 1 km. Further to the west the sedimentary section exceeds 6 km in thickness.

The Arunta Block can be characterized by three tectonic provinces[6,7], each of which underwent separate histories of deformation and metamorphism during early to middle Proterozoic time (Fig. 1). The Southern Arunta Province, between the Redbank Thrust and the northern margin of the Amadeus Basin, is predominantly an amphibolite-grade granitic gneiss, in contrast to the central province which is characterized by felsic and relatively minor mafic granulite that was metamorphosed at depths of ~25 km at ~1,750–1,800 Myr. The Northern Arunta Province consists of low-grade metasediments of amphibolite and greenschist facies. The southern and central provinces are separated by the Redbank Thrust, a 7–10-km-wide east–west-trending zone of anastomosing mylonites which dips northwards at about 45°. The northern and central provinces are separated by large granitoid intrusions and possibly by moderately to steeply dipping faults. Another major structural feature is the Ormiston Thrust Zone some 13 km south of, and running sub-parallel to, the Redbank Thrust. At the northern end of the line, north of the Ngalia Basin, the tectonics becomes complicated by an oblique lineament known as the Weldon Tectonic Zone[5,6]. Apart from this last feature the tectonic strike is primarily east–west and approximately orthogonal to the seismic reflection line.

Deep seismic reflections

All signal processing was carried out using pseudo-true-amplitude techniques, in which energy loss is assumed to occur only

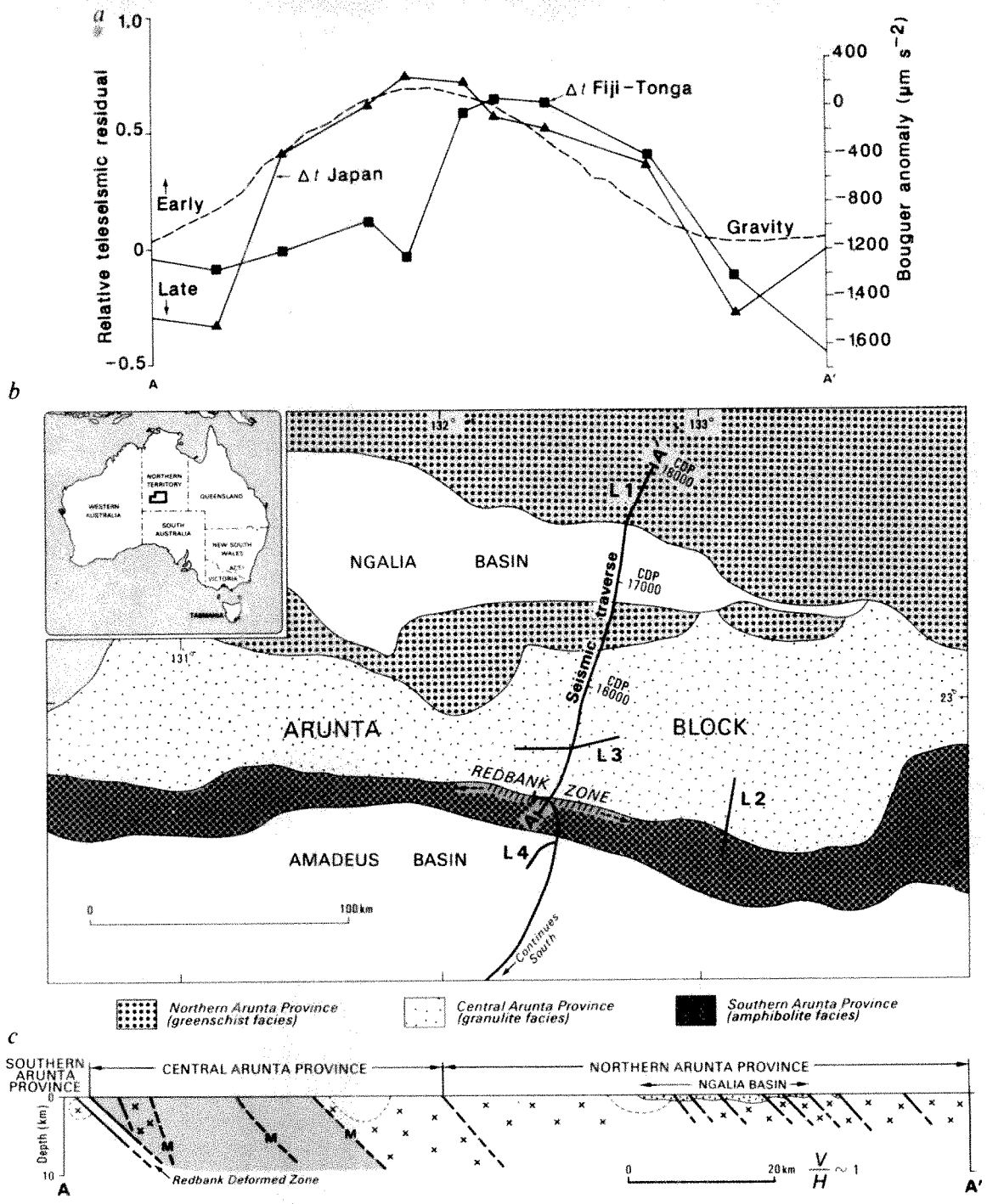


Fig. 1 Location diagram showing the principal tectonic elements of the central Australian region (*b*) and the position of the northern portion of the Central Australian traverse, L1, where it crosses the Arunta Block. Gravity and teleseismic travel-time profiles[1] (*a*) and a schematic geological cross-section (A–A′, shown in *b*) along this traverse are also shown (*c*). The shading schemes used in *b* and *c* are different. The travel-time anomalies are for earthquakes from two different regions: Japan from the north and Fiji–Tonga from the east.

by spherical divergence and losses associated with attenuation are neglected. Major efforts to enhance the deep seismic signal were not required during processing. Figure 2 illustrates the variations in the processed signal throughout the seismic section for a collection of single-shot gathers.

Line drawings of the deep-seismic-reflection section are shown in Fig. 3 and are used here instead of seismic-reflection sections because of the greater clarity of the former and to avoid some of the difficulties of migrating deep seismic data[14]. Each line segment in these drawings represents a coherent seismic reflection seen on the original seismic section. Results obtained with different stacking methods are illustrated for both unmigrated and migrated sections. The migrated sections are produced by migrating each line segment of the line drawin to its time–depth position. The coherency-stacking method (Fi 3*a*) enhances coherent and continuous dipping and flat reflec tors. The corresponding section of migrated line segments i shown in Fig. 3*b*. The energy-stacking method (Fig. 3*c*) empha sizes packets of energy that are not affected by signal polarity and enables an easier correlation of reflections because they ar enhanced above the generally noisy background of the seismi sections[15]. The corresponding line-segment-migrated section shown in Fig. 3*d*. Both migrations were performed with a velo ity–depth function that varied from 5.5 km s^{-1} at shallow depth to 6.2 km s^{-1}.

Other stacking methods have also been used, and they produ

the same general features illustrated in Fig. 3. In addition, most of the deep-reflection events seen on these processed sections can also be identified on the single-fold displays (see, for example, Fig. 2).

The principal features of the line drawings are illustrated in Figs 3 and 4. A general feature of the data is that the strength and depth of penetration of the reflected signal is unusually high for deep seismic data; the signal is still strong to reflection times of 14 s with an unusually high frequency content. Frequencies up to 80 Hz at reflection times of 5–6 s and to 60 Hz at 6–10 s are common, particularly within the Northern Arunta Province. Because conditions around the source were similar to those encountered elsewhere in Australia, the high-frequency content implies that the rocks at depths of 15–20 km have a very high quality factor Q (that is, low attenuation)[16].

The Precambrian Arunta Block exhibits reflections of varying strength throughout the section and is not characterized by the marked non-reflective upper-crustal zone that is seen in similar sections of Precambrian regions elsewhere in the world[17]. Instead, the Arunta section is dominated by northerly-dipping reflections throughout the entire crust, which coincide with surface evidence of thrusts and faults. The surface faults and mylonite zones corresponding to the Redbank Thrust correspond to reflections in the seismic section that can be traced to depths of at least 30–35 km and possibly down to 50 km (Figs 3*b* and 4*a*), dipping at an angle of about 35–40°. Three-dimensional modelling has shown that such faults can generate strong reflected signals[18]. A second band of northerly- but shallower-dipping reflections lies below this feature and its extension to the surface suggests that it corresponds to the Ormiston Thrust, which appears to merge with the Redbank Thrust at mid-crustal levels. Beneath the Southern Arunta Province, the reflectors down to 30 km depth are predominantly sub-horizontal, in contrast to the generally northerly-dipping reflectors (~35°) beneath the central and northern Provinces. This transition in dip coincides with the Redbank Thrust. We interpret the Southern Arunta Province as extending to the underthrust foot wall of this thrust, down to a depth of at least 30 km. The crust north of the Redbank Thrust exhibits further moderately dipping (35–40°) reflections which correspond to surface outcrops of mapped faults or of faults inferred from aeromagnetic surveys. These faults and shear zones extend down to a depth of ~35 km and divide the crust into a series of 'blocks', each of which has similar seismic characteristics and which is bounded by the dipping faults. Seismic-reflection sections of Precambrian crust recorded elsewhere in the world have not exhibited such large numbers of dipping faults and inferred tilted blocks. The shallow zone of low reflectivity (<2 s travel time) seen to the north of the Redbank Thrust coincides with mapped outcrops of granitoid and granitic gneiss, and this whole region is interpreted as granitic material. Sub-horizontal reflections occur at the base of this granitic zone, as well as within the crustal blocks (Fig. 3*b*), and are attributed to compositional layering. They can, therefore, be used as marker horizons which indicate the present attitude of the original crustal layering. Within some blocks these sub-horizontal events are truncated against the dipping faults, suggesting that the deformation of the crust occurred by movement along the faults, with little or no rotation of the crustal blocks.

Deep-crustal reflections in the migrated sections are more prominent beneath the northern and southern provinces than beneath the Central Arunta Province (Figs 3*b*, *d* and 4*a*). In particular, the two migrated sections beneath the central province exhibit a relatively transparent zone at about 25–30 km depth, where the density of reflections is comparable to those usually seen in other sections at depths greater than 35–40 km. Energy is received from reflections beneath this transparent zone, indicating that this region is seismically non-reflective rather than being a region of high attenuation. The continental crust–mantle boundary is often defined in seismic-reflection

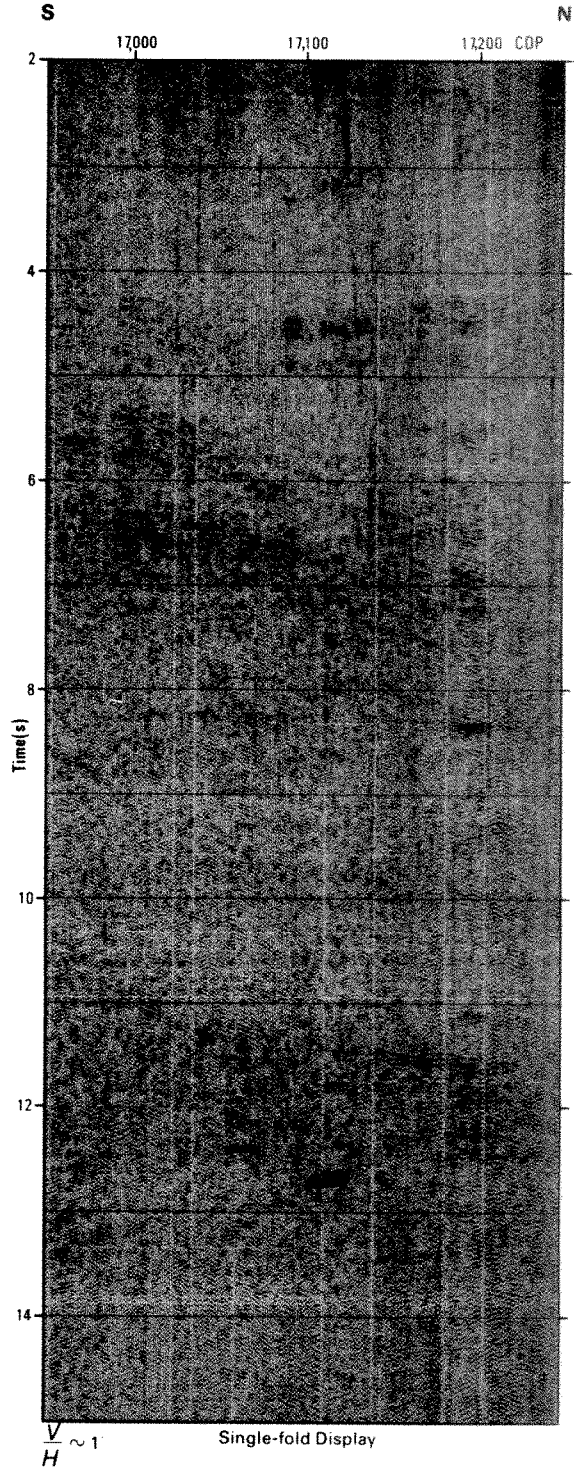


Fig. 2 Portion of a single-fold display, without gain correction, from within the Northern Aunta Province. This is a pseudo-true-amplitude section and illustrates the strength and continuity of the deep signals.

sections as the base of a zone of strong, deep and layered reflections overlying a non-reflective region corresponding to the upper mantle[19,20]. Beneath the northern part of the Arunta Block, the appropriate choice for this boundary would be at about 35–40 km depth but it is less well defined south of the Redbank Thrust. One interpretation is that this boundary occurs at the base of the lowest reflections that are seen at about 50 km depth (Figs 3*b* and 4*a*); this indicates a thicker-than-normal crust and a significant variation in the crustal thickness between the southern and northern provinces. Sediments in the Amadeus

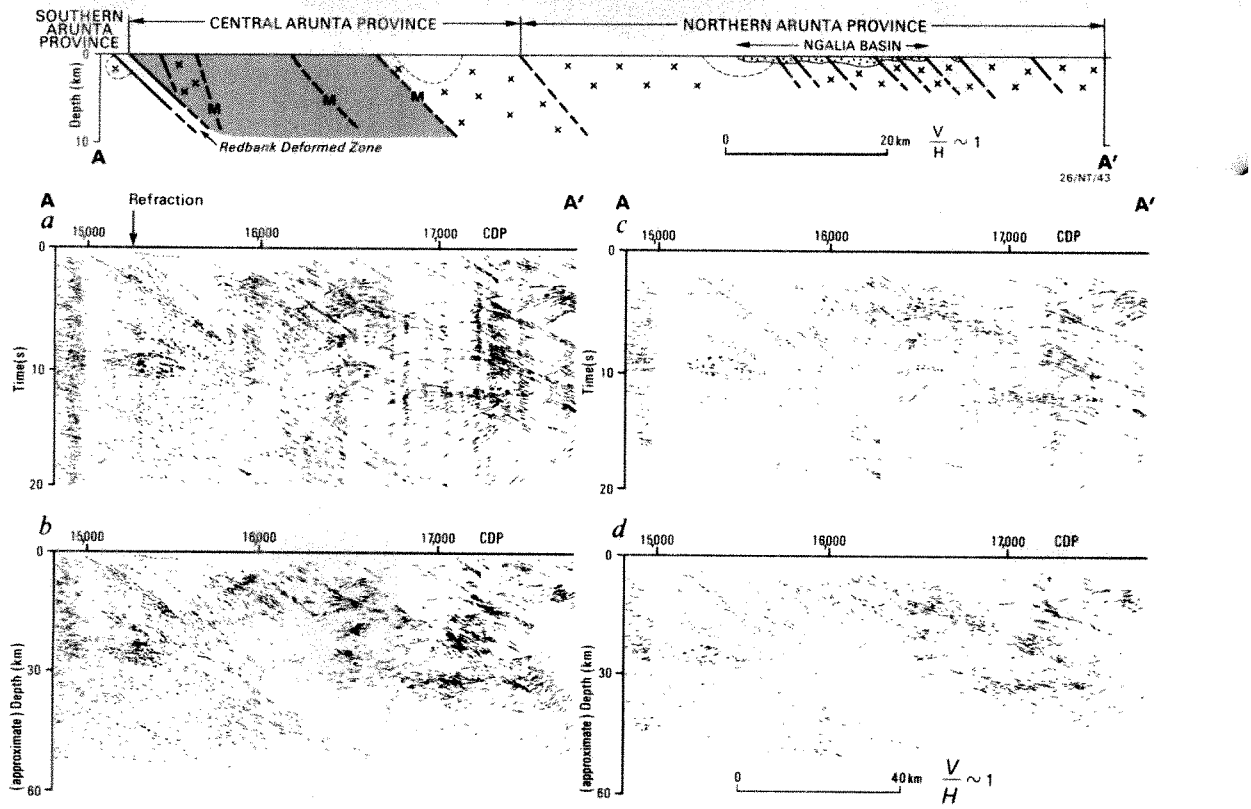


Fig. 3 Unmigrated and migrated line drawings of the seismic-reflection section from the Central Australian traverse. *a*, Unmigrated coherency stack. *b*, Line-migrated version of *a*. *c*, Unmigrated energy stack. *d*, Line-migrated version of *c*.

Basin, south of the Redbank Thrust, have a maximum thickness of 10–14 km and the gravity, teleseismic travel-time anomalies and deep-crustal-reflection data do not indicate any discontinuity in the deep-crustal structure across the basin margin; the interpretation that the base of the crust is at 50 km depth therefore implies that the sediments have been deposited on a crust of approximately normal thickness. This is consistent with a predominantly compressional regime during the later part of the basin formation process. The large negative gravity anomalies, the late teleseismic travel-time arrivals (Fig. 1) across the northern part of the Amadeus Basin and the southern part of the Arunta Block south of the Redbank Zone, and an east–west seismic reflection profile, all favour a crustal thickness of more than 50 km in this region. We therefore interpret the reflection record beneath the footwall of the Redbank Thrust as a broad crust–mantle transition zone of 15–20 km thickness extending down to a depth of at least 50 km. Beneath the Northern Arunta Province this transition zone is considerably thinner and occurs at a shallower depth. The seismically transparent zone immediately north of the Redbank Thrust is interpreted as a region of predominantly mantle material emplaced by major thrusting on the Redbank and Ormiston Thrusts.

Figure 4*b* illustrates the crustal structure derived from the teleseismic travel-time anomalies for the part of the Arunta Block corresponding to the extent of the seismic line. The model has poor resolution north of the Ngalia Basin. The principal requirements of this data set are a zone of relatively high-velocity material dipping steeply northwards such that its continuation to the surface coincides approximately with the Redbank Thrust and the granulite terrain to the north of it, and relatively low-velocity material in the form of a down-thrust wedge to the south of the Redbank Thrust[10]. These features are consistent with the reflection data.

Gravity modelling

The Bouguer gravity anomalies along the seismic line are characterized by a relatively long-wavelength variation of ~1,400 μm s^{-2} peak-to-trough amplitude. The free-air anomalies exhibit a very similar variation, and conventional models of isostatic compensation fail to explain these observations[21]. Near-surface variations in crustal densities of surface outcrop rocks range from 2.5 g cm^{-3} for the Palaeozoic sediments in the basin to 2.95 g cm^{-3} for the high-grade granulite facies rocks north of the Redbank Thrust[10]. If such lateral variations persist down to 10 km depth they would explain about 50% of the observed magnitude of the gravity anomalies. However, the wavelength predicted for these anomalies is considerably less than that observed, and the observed maximum anomaly is offset from the surface exposure of high-density granulites by more than 50 km. Furthermore, there are no significant short-wavelength changes observed across either the Redbank Thrust or the Amadeus Homocline, despite the existence of density contrasts across these surfaces[10]. Overall, these observations suggest a deep origin for the major part of the observed gravity field over the southern part of the Arunta Block. Figure 5 shows the gravity field predicted by the model, using density values based on surface-rock exposure[9,10]. Agreement with the observed field is satisfactory and could be further improved by minor adjustments to the boundaries that separate regions of different densities or by introducing density gradients within the regions. Most importantly, the model makes good predictions of both the amplitude and the wavelength of the observed field.

A tectonic model for the Arunta Block

The deep-seismic-reflection data favour a tectonic model in which the major deformation within the Arunta Block occurred along faults and shear zones that extend from the surface to the crust–mantle boundary (that is, a 'thick-skinned' model). In particular, there is no seismic evidence for mid-crustal ramps branching from a relatively shallow-dipping sole thrust, which is a principal characteristic of the thin-skinned models[22]. In this sense, the Arunta structure is comparable to both the Flannan and Outer Isles Thrusts of Scotland[23], the Pyrenees of Europe[2],

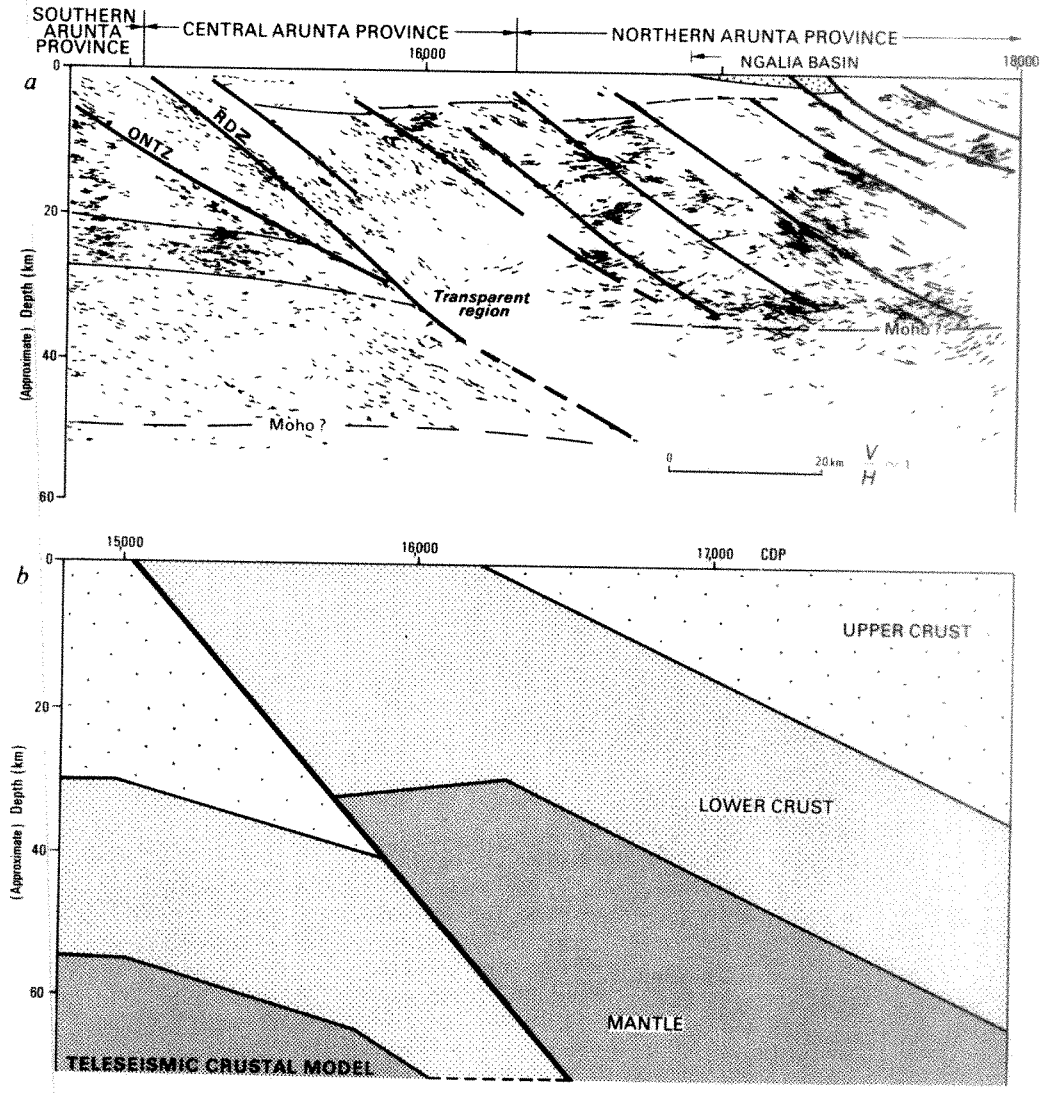


Fig. 4 Interpreted crustal structure of the Arunta Block. RDZ refers to the Redbank Deformed Zone and ONTZ to the Ormiston Nappe and Thrust Zone. *a*, Deep-seismic-reflection model. *b*, Teleseismic crustal model, based on the observed teleseismic travel-time data[1].

the Grenville Front of Canada[5] and, to a lesser degree, the Wind River Thrust of the USA[24], but it differs in that these latter structures do not exhibit the seismically transparent material on the lower part of the hanging wall. None of the Flannan, Outer Isles or Wind River Thrusts are associated with a major gravity anomaly. It is emphasized that the above remarks refer to the central part of the Arunta Block and Amadeus Basin, and that in an intracratonic environment the deformations along the eastern and western limits may differ in significant ways from this model.

Structural, metamorphic, and isotope data reveal that the Arunta Block has had a complex history. In particular, Rb/Sr and $^{40}Ar/^{39}Ar$ isotope data imply different evolutionary histories for the blocks north and south of the Redbank Thrust before 1,450 Myr and possibly until 1,100 Myr (ref. 9). The overall character of the seismic reflections changes across this thrust and this may be indicative of fundamental differences in crustal composition of the two regions, supporting the interpretation that the Redbank Thrust is a major crustal boundary. Furthermore, the isotope data and the metamorphic grade of the mylonites indicate that the granulites north of the Redbank Thrust were subjected to major uplift (~15 km) during the Alice Springs orogeny, particularly during the last phase of the Amadeus Basin formation which produced the upturned and overthrust northern margin (the Amadeus Homocline). Argon-loss data constrain this uplift at between 300–400 Myr, with the movements on the faults decreasing in age from north to south between the Redbank and Ormiston Thrusts[9].

Teleseismic travel-time residuals[10] favour models in which low-velocity crust is underthrust beneath overriding lower crust and mantle of the Central and Northern Arunta Provinces down to a depth in excess of 50 km (Fig. 4). The isotope evidence shows that a substantial part of this structure developed at a time leading up to and including the Alice Springs orogeny, producing a stacking or imbrication of the crust and the exposure of lower-crustal material north of the Redbank Zone. Displacement of the inferred granitic layer and the deflection of sub-horizontal reflectors against the fault or shear zones suggest that the faults north of the Redbank Thrust acted as 'lag' faults during the final stages of movements.

This model generates a number of questions. In particular, one can ask what are the driving forces that have produced this structure, how such a structure, obviously out of isostatic equilibrium, has been maintained for the past 300 Myr, and how these conclusions relate to other thick-skinned regions. The stress differences associated with the present structure are of the order of 100–150 MPa (ref. 10) and the stress fields associated with the forces that produced the structure must have been at least of this magnitude. The isostatic response is anisotropic. The east–west response is consistent with models of local or regional isostatic compensation, whereas the north–south response implies compressional in-plane stresses of the order mentioned[21]. The major movements on the Redbank Thrust before and during the Alice Springs orogeny indicate that compressional forces operated at this time, and the geological setting of central Australia indicates that this occurred within an intracratonic environment rather than at a plate margin. The timing of the Alice Springs orogeny coincides with the Kanimblan

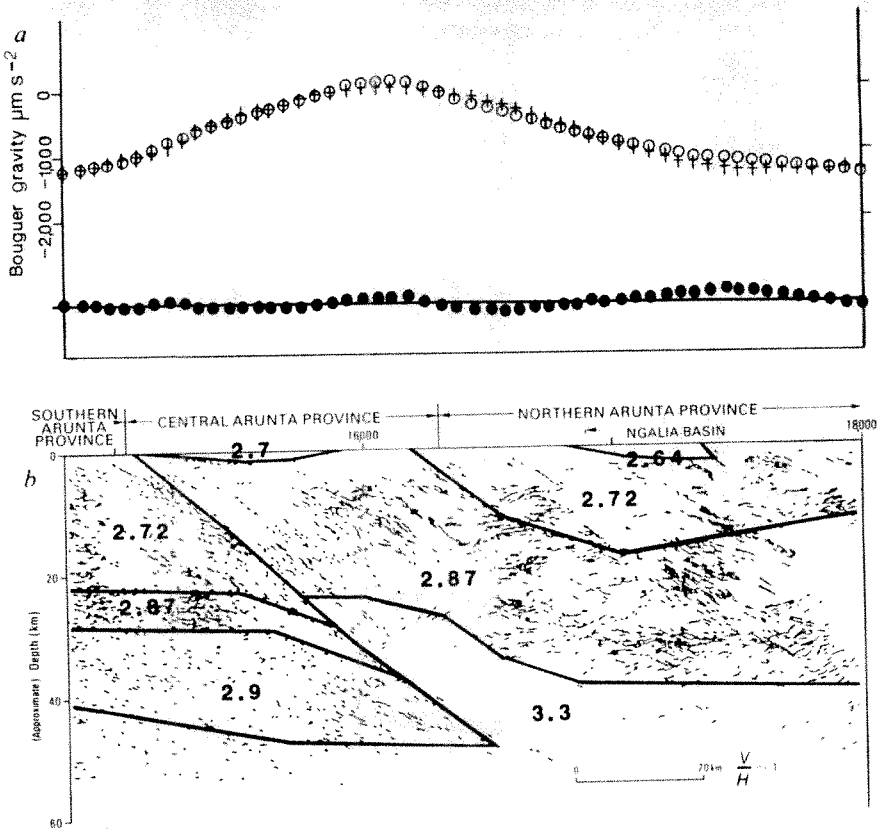


Fig. 5 *a*, Observed and computed gravity profiles along the deep seismic line. The computed profile is based on the interpreted deep-seismic-reflection model (*b*) for the Arunta Block. In *a*, crosses represent the observed profile, open circles represent the computed profile and filled circles represent the residual profile ± 150 μm s^{-2}. Numbers superimposed on *b* are the densities in g cm^{-3} assumed in the gravity calculations.

orogeny of eastern Autralia[25]; this suggests that the intracratonic movement may have been primarily the consequence of the lithosphere acting as a stress guide to forces operating at and shaping the plate margin some 1,000 km or more distant from the Redbank Zone[26]. The implication is that stress differences of 100 MPa and more can be transmitted by the lithosphere over distances equal to the dimensions of tectonic plates and that the evolution of intracratonic tectonic features such as basins may be driven by forces external to the area. Evidence for this can also be seen elsewhere, for example, in the relation between the tectonics of north-west Europe and the latest Cretaceous and Tertiary Alpine orogeny[27].

Once formed, stress relaxation would be expected to lead to isostatic rebound so that the area would reach some state of mechanical equilibrium. There is, however, surprisingly little evidence for major re-adjustment with time. The maximum gravity anomaly is associated with a very mild topographic low and the minimum anomaly is associated with a regional topographic high over the northern part of the basin, suggesting that some rebound has occurred[10]. At the time of the last orogenic event the area would have carried a significant topographic load created by the upthrust crust and by the synorogenic sediment loads deposited on the flanks of the thrust. A study of fission-track ages[28] and isotope evidence[9] points to a removal of up to 4 km of material from both the northern part of the Amadeus Basin and the southern part of the Arunta Block. Thus, immediately after the orogenic event, the region may have been close to isostatic equilibrium, but as erosion occurred the crust did not respond fully to the redistribution of surface loads. The probable explanation is that part of the north–south horizontal compressive force that led to the thrusting was still present after termination of the orogeny, at which time the lithosphere strengthened, locking the sub-surface structure and producing an increasingly regional response to the lateral variations in surface and internal loads[22]. We note that two major earthquake sequences occurred recently in central Australia, one to the south, in the Musgrave Block[29], and the other about 350 km north of the Ngalia Basin[30], both of which indicate that the present lithospheric stress field is one of north–south compression.

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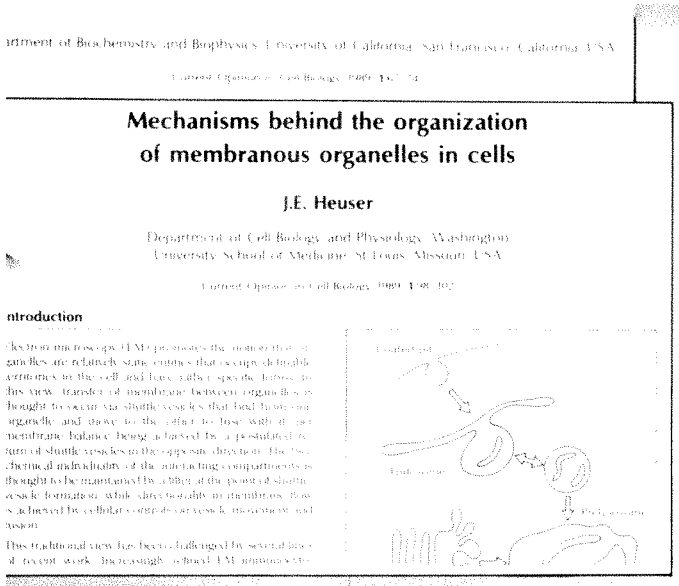
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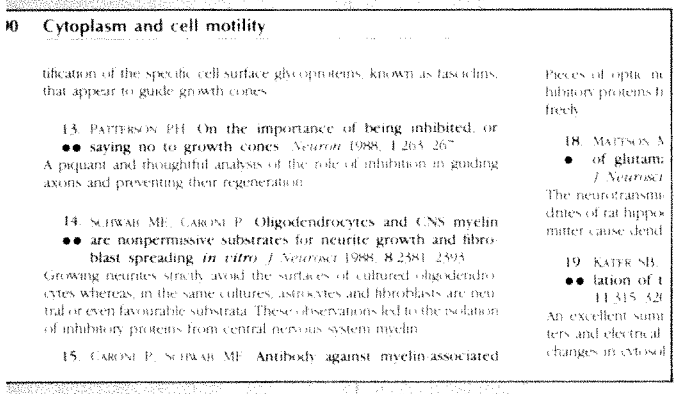


Mechanisms behind the organization of membranous organelles in cells

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Introduction

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10 Cytoplasm and cell motility

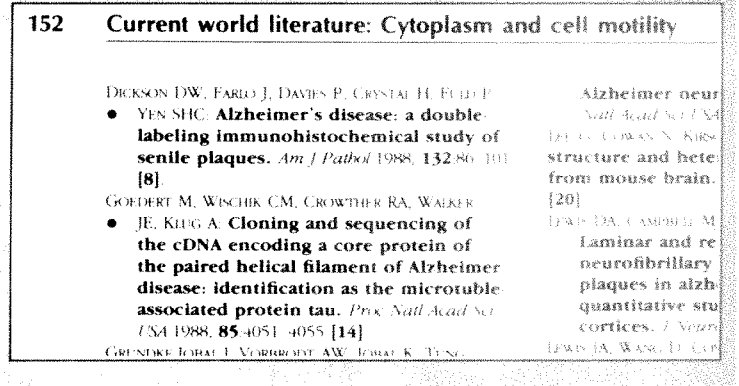
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A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis

Carol W. Greider* & Elizabeth H. Blackburn†

* Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724, USA
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The telomerase enzyme of Tetrahymena *synthesizes repeats of the telomeric DNA sequence TTGGGG de novo in the absence of added template. The essential RNA component of this ribonucleoprotein enzyme has now been cloned and found to contain the sequence CAACCCCAA, which seems to be the template for the synthesis of TTGGGG repeats.*

NUCLEIC acid synthesis reactions are usually template directed. Exceptions include the reactions of terminal deoxynucleotidyl transferase, which adds any deoxynucleotide in a random order onto the 3′ end of a polynucleotide chain[1], poly(A) polymerase which adds rAMP residues to the 3′ end of RNA[2], and tRNA nucleotidyl transferase which adds the sequence CCA to the 3′ end of tRNAs[3]. In addition, we have discovered the enzyme telomerase which synthesizes tandem repeats of the telomeric DNA sequence TTGGGG to the 3′ end of telomeric primers without any added template[4,5].

Telomeres from such diverse organisms as ciliates, yeasts, plants, and mammals are made up of a variable number of tandemly repeated, simple (G+C)-rich sequences[6–12]. In all telomeres examined, there is a strand-specific sequence bias such that the G-rich strand always runs 5′ to 3′ towards the end of the chromosome. As the number of tandem repeats on the end of any given chromosome is variable[13,14], restriction fragments containing telomeres have a characteristic 'fuzzy' appearance in agarose gel electrophoresis. We have proposed that this length heterogeneity arises from a dynamic equilibrium established between the incomplete replication and/or degradation of the chromosome end and the *de novo* synthesis of telomeric sequences by telomerase[4,12–14].

The *Tetrahymena* telomerase extends the free TTGGGG-OH 3′ telomere strand by the addition of further TTGGGG repeats. Both the repeated sequence motif and the orientation of the G strand have been conserved throughout evolution, suggesting that telomerase-like enzymes may be found in all eukaryotes. The presence of telomerase would explain several observations about telomeres. First, when *Tetrahymena* or *Oxytricha* telomeric sequences are introduced into yeast, telomeric sequences characteristic of yeast are added to them[12,15,16]. Second, when trypanosomes or *Tetrahymena* are kept in continuous log-phase growth, there is a net increase in telomere length[13,17]. In *Tetrahymena* this increase involves all of the telomeres in the cell and is due to the addition of telomeric TTGGGG repeats[13,14]. Finally, telomeric sequences from a number of different organisms are maintained as perfect tandem repeats and do not diverge through recombination, unlike most simple repeated sequences in the nuclear genome[18–20] and mitochondrial telomeres[21,22]. This sequence maintenance can be explained by the continual synthesis of new perfect telomeric repeats.

Recently, telomerase activities have been isolated from the nuclei of both the hypotrichous ciliates *Oxytricha* and *Euplotes* (ref. 23; D. Shippen-Lentz and E. Blackburn, submitted). These enzymes synthesize repeats of the telomeric sequences of those organisms, TTTTGGGG, in an analogous way to the *Tetrahymena* telomerase. Together with the conserved structure of telomeres, these results further indicate that telomerase enzymes exist throughout eukaryotes.

We showed previously that the telomerase of *Tetrahymena* is a ribonucleoprotein (RNP) enzyme with essential RNA and protein components[5]. Because the reactions catalysed by many RNPs involve base pairing of the RNA components[24–28], the requirement of an RNA component in the *Tetrahymena* telomerase suggested that the synthesis of TTGGGG repeats might be specified by the RNA[5]. We report here the identification of the essential RNA component of telomerase and show that this RNA contains the sequence CAACCCCAA which could serve as a template for the synthesis of TTGGGG repeats.

Cloning and characterization of 159 RNA

We previously showed that small RNAs of approximately 159 bases and 80 bases co-purified with the telomerase RNP over five column purification steps[5]. Further purification of the telomerase over several other column series indicated that the RNA of about 159 bases (159 RNA) was the only one to reproducibly co-purify with the enzyme activity (data not shown). We directly sequenced the 159 RNA using both sequence-specific RNases and dideoxynucleotide primer extension techniques. Southern blot analysis of *Tetrahymena* macronuclear DNA, using oligonucleotide probes derived from the RNA sequence, indicated that there was a single gene for the 159 RNA in the macronuclear genome. A 2 kilobase (kb) *Hin*dIII fragment containing the gene was cloned from macronuclear DNA. The sequence of a 400-base region of the clone containing the 159 RNA coding sequence is shown in Fig. 1*a*. The most notable feature of the gene is the presence of the sequence 5′ CAACCCCAA 3′ from positions 43 to 51 within the coding region. This sequence could serve as a template for the addition of TTGGGG repeats *in vitro*.

Three ribonucleotide residues at positions 46, 47 and 48 in the 159 RNA were not cut by any of the ribonucleases used in the direct RNA sequencing, indicating that these bases might be modified, although primer extension by reverse transcriptase was unimpeded at these positions. Several different base modifications in tRNAs are known to interfere with ribonuclease cleavage[29]. Ribose 2′-*O* methylation inhibits both RNase and base hydrolysis of RNA by blocking the formation of the cyclic phosphate intermediate[30]. As the base hydrolysis ladder was complete at these positions in the telomerase RNA (data not shown), this type of modification can be ruled out. Both secondary structure modelling of the telomerase RNA (I. Tinoco, personal communication) and data presented below indicate that this region of the RNA is not in a tightly base-paired structure; hence structural interference was probably not responsible for the inability of RNases to cut at these positions. Although the nature of these possible base modifications is not known, it is intriguing that they lie within the CAACCCCAA sequence.

Several features of the 159 RNA gene suggest that it is transcribed by RNA polymerase III. First, the length of the 159 RNA is heterogeneous. Using high-resolution polyacrylamide gel electrophoresis we separated a cluster of four bands and sequenced the two prominent species in the middle. These two species differed only in the number of U residues at the 3′ end. In the sequence of the cloned gene this 3′ end of the RNA falls

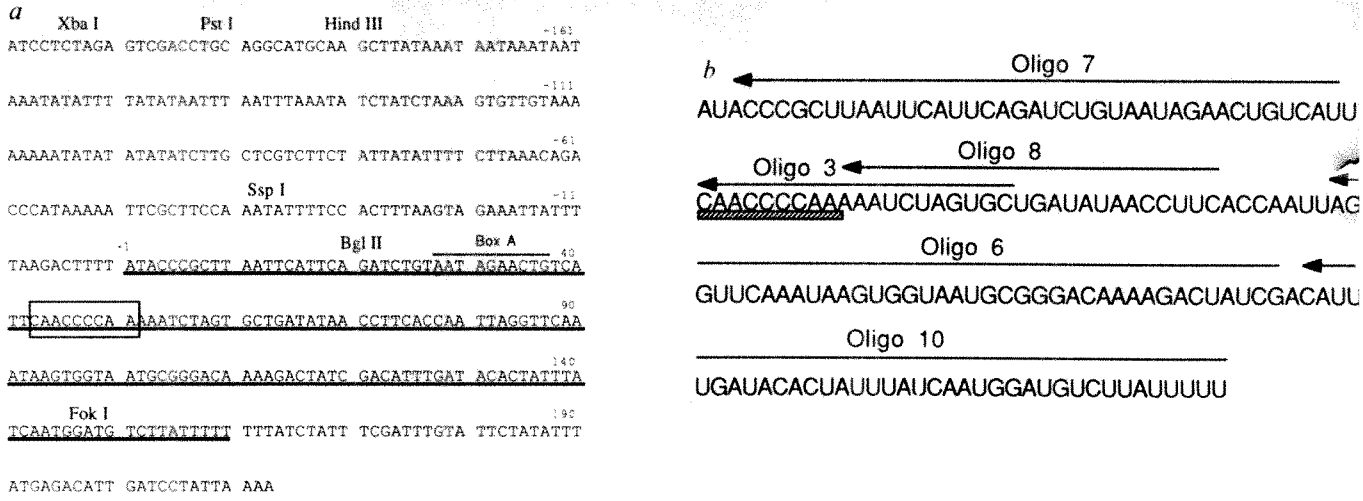


Fig. 1 *a*, Sequence of the telomerase RNA gene. The first 30 nucleotides of the pUC119 polylinker up to the *Hin*dIII site used for cloning are shown for orientation along with 370 bases of the non-template strand of the telomerase RNA gene. The region corresponding to the RNA is underlined. The start site of transcription as determined by dideoxy primer extension sequencing is labelled +1 and the 3′ end of the RNA as determined by direct RNA sequencing is shown within the run of eight T residues near position 160. All of the six-base recognition restriction enzymes which cut in the *Tetrahymena* DNA are shown above their recognition sites. A region of similarity with the consensus Box A for polymerase III genes is shown at positions 28–37. The sequence CAACCCCAA within the RNA gene which may provide the template for the addition of TTGGGG sequences *in vitro* is shown in the box at position 43. *b*, The oligonucleotides (oligos) used for cloning, sequencing and RNase H experiments are shown above the telomerase RNA regions to which they hybridize. The region of the RNA containing the potential template is underlined with a hatched bar. Oligonucleotides 1–6 were made using the sequence information obtained by partial enzymatic RNase sequencing. Oligonucleotides 1, 2, 4 and 5 were shown to contain significant mismatches with the correct sequence obtained by DNA sequence analysis. Oligonucleotides 7, 8 and 10 were synthesized using DNA sequence information. Only the oligonucleotides that were shown to hybridize to the 159 RNA are shown. The sequence and name of all the oligonucleotides used throughout this paper are listed from 5′ to 3′. Oligo 2, TTCTGTTGCAAAATCTGAATGAAT. Oligo 3, GCACTAGATTTTTGGGGTTG. Oligo 5, TTGTTTGAACCTGATT-GTGAAGGTT. Oligo 6, CGATGGTCTTTTGTCCCGCATTGCCACTTGTTTGAACCT. Oligo 7, ATGACAGTTCTATTACAGATCTGAAT-GAATGAATTAAGCGGGT. Oligo 8, GAAGGTTATCAGCACTAGATTT. Oligo 10, AAAAATAAGACATCCATTGATAAATAGT-GTAT-CAAATG.

Methods. Telomerase S100 preparation (60 ml) was fractionated over a sizing column, heparin agarose, and spermine agarose[5]. An aliquot of the active fraction (30 ml) was digested with proteinase K, phenol extracted and ethanol precipitated; half of the RNA was 3′ end-labelled with [^{32}P] pCp using RNA ligase and half was treated with calf intestinal phosphatase, phenol extracted and labelled with [γ-^{32}p]ATP using polynucleotide kinase. The labelled RNA was run on an 80 cm 15% acrylamide/7M urea gel for 21 hours at 3,500 V. The two major bands corresponding to the 159 and 160 species of RNA were cut out, eluted from the acrylamide and partial enzymatic RNA sequencing was done. The sequence obtained from the 5′ and 3′ labelled RNAs overlapped by 50 bases. Thus the entire sequence was obtained except for a few bases where the sequence data from both 5′ and 3′ labelled RNA was unreadable possibly due to RNA base modifications (see text). Using this sequence information, oligonucleotide 6 was synthesized and used as a primer for dideoxy primer extension RNA sequencing. Eighty-one nucleotides of sequence were obtained and oligonucleotides were then synthesized and used for cloning of genomic copy of the RNA. *Tetrahymena* macronuclear DNA was cut with *Hin*dIII and size fractionated on a 0.8% agarose gel. The region around 2.0 kb was cut out and electroeluted. This DNA was ligated into *Hin*dIII-cut pUC119 plasmid and transformed into DH5α F′. 30,000 transformants were obtained: of these 10,000 were screened by colony hybridization. Replica plates were made of the transformation plates, and duplicate colony lifts onto nitrocellulose were made. The duplicate filters were hybridized in 6x SSC, 4x Denhardts and 0.1% SDS with either oligonucleotide 3 or oligonucleotide 6 at 55 °C for 15 hours. The filters were then washed three times at room temperature in 3x SSC 0.1% SDS for 10 min, and then in 3x SSC 0.1% SDS at 55 °C for 10 min. One clone was obtained which hybridized to both oligonucleotides. The clone was sequenced using double stranded dideoxy sequencing (USB-sequenase kit) with the reverse universal primer and an oligonucleotide internal to the RNA gene as primers.

in a stretch of eight T residues. These properties are typical of RNA polymerase III transcription termination[31]. Second, we were able to label the 5′ end of RNA with [γ-^{32}P]ATP and polynucleotide kinase after treatment with phosphatase, indicat- 5′ end of the 159 RNA was not capped (data not shown). Third, there is a region of similarity to the box A consensus RRYNNARYGG[32] beginning 30 nucleotides downstream of the 5′ end of the 159 RNA.

d(TTGGGG)$_4$ elongation interference

We used RNase H inactivation to establish whether the 159 RNA was an essential component of telomerase. This technique has been used to identify essential RNAs for a variety of RNPs[27,28,33–36]. Telomerase was preincubated with antisense oligonucleotides (Fig. 1*b*) in the presence or absence of RNase H. After the preincubation, the primer d(TTGGGG)$_4$ oligonu-cleotide was added along with [α-^{32}P] dGTP, dTTP and reaction buffer, and the ability of telomerase to synthesize TTGGGG repeats was assayed (Fig. 2). Oligonucleotides 2, 5, 6, 7 and 10, which are complementary to the 159 RNA, had no effect on telomerase activity in either the presence or absence of RNase H. However, oligonucleotide 3 strongly inhibited telomerase activity in either the presence or absence of RNase H, and preincubation with oligonucleotide 8 resulted in an upward shift in the characteristic six-base banding pattern, suggesting this antisense oligonucleotide was itself efficiently priming repeat addition (Fig. 2; see lanes 17–20). In the absence of added RNase H the 159 RNA was intact, indicating that endogenous RNase H was not responsible for the inhibition of telomerase by oligonucleotide 3 (Fig. 4 and data not shown). Although in this initial experiment the effects of RNase H addition were not definitive, the observations that oligonucleotides 3 and 8, which hybridize across, and near to, the CAACCCCAA sequence respectively, specifically affected telomerase activity suggested the 159 RNA is involved in telomerase activity.

Northern analysis of RNA extracted from active telomerase fractions probed with oligonucleotides 3 and 8, showed only one band at 159 nucleotides (data not shown). Thus, these oligonucleotides do not share extensive sequence complementarity with other RNAs in the enzyme fractions used throughout this work.

The inhibition of telomerase activity by preincubation with

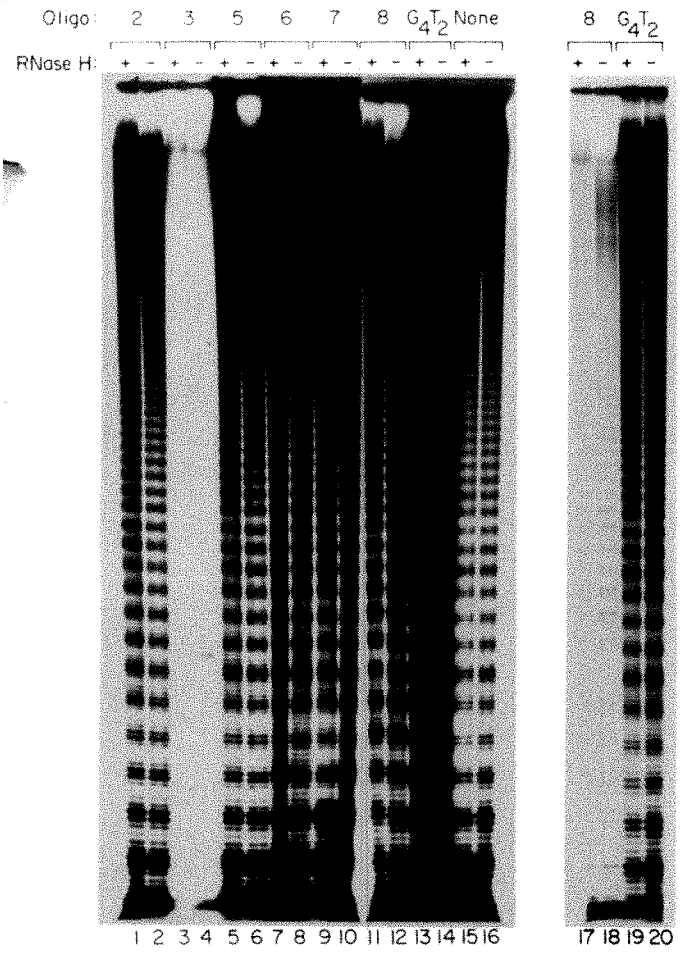


Fig. 2 Treatment of telomerase with antisense oligonucleotides and RNase H. The oligonucleotides indicated were preincubated with telomerase in the presence (+) or absence (−) of RNase H. The ability of telomerase to elongate added d(TTGGGG)$_4$ primer was then assayed. The oligonucleotides added in the preincubation are indicated above the lanes. G_4T_2 is d(TTGGGG)$_4$. Lanes 17–20 show a lighter exposure of lanes 11–14 to indicate better the upward shift in the banding pattern when oligonucleotide 8 is included in the preincubation.

Methods. Telomerase which had been purified over a sizing column and heparin agarose was used[5]. Antisense oligonucleotide (2 μl) at 0.4 μg μl^{-1} was added to 10 μl of telomerase, followed by either 2 μl of RNase H at 2U μl^{-1} (BRL) or 2 μl of TMG + 0.1 M NaCl (TMG, 10 mM Tris *p*H 8.0, 1 mM $MgCl_2$, 10% glycerol) were added. The samples were preincubated at room temperature for 30 min and then 6 μl of TMG + 0.1 M NaCl and 20 μl of 2x reaction mix was added. The 2x reaction mix contained: 0.1 μg d(TTGGGG)$_4$, 200 μM dTTP, 200 mM NaOAc, 100 mM Tris *p*H 8.0, 10 μCi [^{32}P]dGTP at 400 Ci mmol^{-1}. Reactions were carried out at 30 °C for 60 min, the mix then phenol extracted, ethanol precipitated and run on a 6% polyacrylamide sequencing gel.

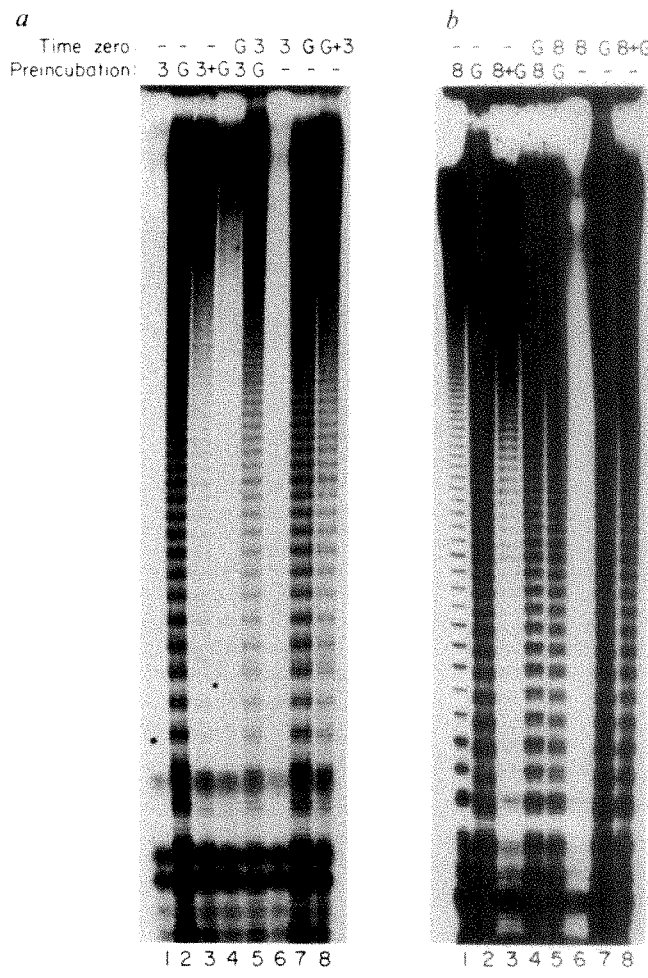


Fig. 3 *a*, Competition between oligonucleotide 3 and d(TTGGGG)$_4$. Telomerase was preincubated at room temperature with the oligonucleotides indicated above the lanes. G is d(TTGGGG)$_4$. At time zero, reaction buffer was added and the reaction was carried out as described in the Fig. 2 legend. The oligonucleotides indicated were added at time zero. *b*, Competition between oligonucleotide 8 and d(TTGGGG)$_4$. The oligonucleotides indicated above the lanes were added in the preincubation. G is d(TTGGGG)$_4$. At time zero the oligonucleotides indicated were added. Darker exposure of the autoradiogram showed a banding pattern in lane 6 similar to that in lane 1.

Methods. 20 μl of telomerase purified as described in the Fig. 2 legend was preincubated with 1 μl of each oligonucleotide at 0.4 μg μl^{-1} or with 1 μl of water as indicated. Preincubation was at room temperature for 30 min. At time zero 20 μl of 2x reaction mix containing 1 μl of the appropriate oligonucleotides or no oligonucleotide was added and the standard activity assay was carried out (see Fig. 2 and ref. 5).

oligonucleotide 3 even in the absence of RNase H suggested that this oligonucleotide might interfere with the access of d(TTGGGG)$_4$ to telomerase. To test for specific inhibition, we reversed the order of addition of the two oligonucleotides. When oligonucleotide 3 was used in the preincubation and the d(TTGGGG)$_4$ was added at the beginning of the elongation reaction (referred to as time zero) there was the same inhibition as in Fig. 2. If d(TTGGGG)$_4$ was added first to the preincubation and then oligonucleotide 3 was added at time zero, there was no inhibition however. When oligonucleotide 3 and d(TTGGGG)$_4$ were added together, an intermediate level of inhibition was seen (Fig. 3*a*). Thus, inhibition by oligonucleotide 3 is a specific effect and its properties are consistent with competition with d(TTGGGG)$_4$.

As oligonucleotide 3 contains the sequence TTGGGGTTG at its 3′ end, its interference with d(TTGGGG)$_4$ elongation could simply be due to its sequence similarity with the d(TTGGGG)$_4$ primer. We also found that oligonucleotide 8, which does not contain any TTGGGG sequence, is itself efficiently elongated by telomerase (Fig. 3*b*). The addition of oligonucleotide 8 alone resulted in the synthesis of a repeat pattern that was shifted upward relative to the pattern primed by d(TTGGGG)$_4$. The repeat pattern visualized by this gel assay is dependent on the sequence and length of the input primer oligonucleotide[5]. Thus, the upward shift in the banding pattern distinguishes the products primed by addition to oligonucleotide 8 from those primed by d(TTGGGG)$_4$. When oligonucleotide 8 was added in the preincubation and d(TTGGGG)$_4$ was added at time zero, the main products had the oligonucleotide 8-primed pattern. In contrast, when the d(TTGGGG)$_4$ oligonucleotide was added

first and oligonucleotide 8 was added at time zero, the characteristic d(TTGGGG)$_4$ primed pattern was seen. Simultaneous addition of the two oligonucleotides, either in the preincubation or at time zero, resulted in a mixture of the two patterns indicating that both oligonucleotides were being elongated under these conditions. As oligonucleotide 8 was the only oligonucleotide with a non-telomeric sequence, of 14 tested, that was efficiently elongated by telomerase (see also ref. 5; data not shown), we suggest that the base pairing of oligonucleotide 8 to the 159 RNA adjacent to the CAACCCCAA sequence allows its elongation by telomerase.

Inactivation by cleavage of 159 RNA

The interaction of oligonucleotides 3 and 8 with telomerase *in vitro* suggested that the 159 RNA is a component of telomerase. To investigate further the requirement for this RNA, we sought conditions under which the effects of cleavage of the 159 RNA could be assayed. In RNase H inactivation experiments oligonucleotides 2, 6, 7 and 10, complementary to the 159 RNA, did not inhibit telomerase activity or cause cleavage of the 159 RNA by RNase H (Fig. 2 and data not shown). To assay the effects of oligonucleotide 3 and RNase H, we had first to relieve the inhibition by oligonucleotide 3. We pelleted the telomerase in an ultracentrifuge after preincubation with the test oligonucleotide and RNase H. The enzyme activity of each resuspended pellet was then assayed (Fig. 4*a*) and the RNA examined for evidence of 159 RNA cleavage (Fig. 4*b*). There was no effect on either telomerase activity or the 159 RNA after preincubation with oligonucleotide 7 in either the presence or absence of RNase H. After preincubation and subsequent removal of oligonucleotide 3 in the absence of RNase H, both telomerase activity and the 159 RNA were intact. Preincubation with oligonucleotide 3 in the presence of RNase H however, inhibited telomerase activity and caused cleavage of the 159 RNA. In a separate experiment, we used primer extension to map the sites at which RNase H cuts in the telomerase RNP (Fig. 5). Although in this experiment cutting by RNase H was incomplete, oligonucleotide 3 directed cutting at several positions along its region of complementarity to the 159 RNA, including nucleotides in the CAACCCCAA sequence.

Oligonucleotide 8 also directs RNase H cutting of the 159 RNA; cutting with this oligonucleotide, even when apparently complete, did not however inactivate the telomerase (data not shown). Oligonucleotide 8 cannot base pair in the CAACCCCAA sequence and accordingly all of the cut sites mapped by primer extension are 3′ to the potential template sequence (Fig. 5). Although the ability of telomerase to function after oligonucleotide 8-directed cleavage of the RNA was surprising, it is not unprecedented. The signal recognition particle retains its microsomal translocation activity after micrococcal nuclease digestion which removes up to 150 nucleotides of the 300 nucleotides in the essential 7SL RNA[37,38]. In addition, the 25S rRNA from mature ribosomes of insects is found naturally cleaved into two halves with some sequences missing[39,40]. Furthermore, in *Crithidia*, the 25S RNA is found cleaved into five pieces[41] and in *Chlamydomonas* both the large and small rRNAs are processed into multiple pieces that are held together by intermolecular base pairing[42]. Finally, deletion and replacement analysis of the essential RNA component of the yeast U2 small nuclear RNP has shown that not all regions of this RNA are essential for activity *in vivo*[43]. The specificity of telomerase inactivation by oligonucleotide 3-directed cutting within the CAACCCCAA sequence, compared with the cutting near this sequence directed by oligonucleotide 8, is evidence for the important nature of the CAACCCCAA sequence in telomerase activity.

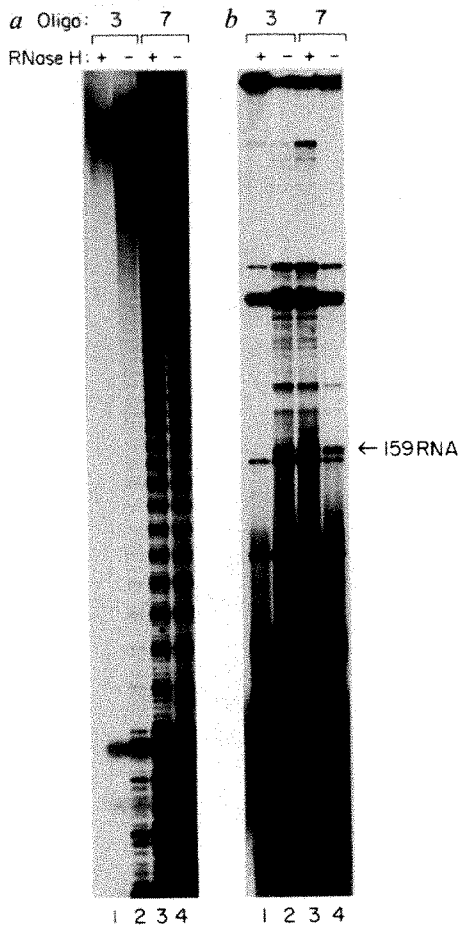


Fig. 4 *a*, The activity of telomerase which had been pretreated with various oligonucleotides in the presence or absence of RNase H and then pelleted in a ultracentrifuge to remove excess oligonucleotide. RNase H was added during the preincubation to the reactions shown in lanes 1 and 3, but not in lanes 2 and 4. The oligonucleotides used in the preincubation are indicated above the lanes. The low level of activity seen in lane 2 relative to lanes 3 and 4 is due to the inhibitory effects of residual oligonucleotide 3 in these fractions. The dark material seen at the top of the gel in lane 1 is due to labelling of high relative molecular mass nucleic acid by some enzyme in the partial purified fraction. More highly purified telomerase fractions do not show this label accumulation. *b*, RNA from the pellets in *a* was extracted, 3′ end-labelled with [^{32}P] pCp and run on a sequencing gel. The lanes are as in *a*. The position of the 159-base telomerase RNA is indicated with an arrow.

Methods. 200 μl of telomerase purified as described in the Fig. 2 legend was preincubated with 6 μl of the appropriate oligonucleotide at 1 μg μl^{-1} in the presence or absence of 5 μl of RNase H at 2U ul^{-1}. Preincubation was at room temperature for 15 min. The extracts were then transferred to Beckman microfuge tubes and spun at 75,000 r.p.m. in a Beckman TLA-100.3 rotor at 4 °C for 3 hours. The supernatant was removed carefully and the pellet was resuspended in 100 μl of TMG+0.1 M NaCl. 20 μl was immediately assayed in the standard activity assay (see Fig. 2 and ref. 5). To the remaining 80 μl, 10 μg of proteinase K was added and the mix was incubated at room temperature for 15 min. The preparation was then phenol-extracted, ethanol-precipitated, 3′ end-labelled with pCp as described[5] and run out on a 6% polyacrylamide/7 M urea sequencing gel.

Discussion

CAACCCCAA sequence may provide a template for TTGGGG repeat synthesis. We have identified and cloned the gene for an essential RNA component of telomerase. Several lines o evidence indicate that the synthesis of TTGGGG repeats i directed by an RNA template. First, the only RNA which repro ducibly co-purified over numerous columns contains th sequence CAACCCCAA. Second, oligonucleotide 8 whose 3 end base pairs just downstream from the CAACCCCA sequence is efficiently elongated by telomerase, whereas all othe

non-telomeric sequences are not. Third, oligonucleotide 3, which is complementary to the telomerase RNA sequences up to and including the CAACCCCAA sequence, inhibits telomerase activity. Finally, cleavage by oligonucleotide 3 and RNase H within the CAACCCCAA sequence specifically inactivates telomerase activity. These observations suggest that the CAACCCCAA sequence of the telomerase RNA is in the active site of the RNP and that this sequence provides a template for synthesis of TTGGGG repeats.

Primer elongation by telomerase can result from two modes of primer recognition. Preincubation of telomerase with $d(TTGGGG)_4$ and RNase H did not inhibit enzyme activity (Fig. 2) or result in cleavage of the telomerase RNA, whereas there was RNA cleavage when $d(TTGGGG)_4$ was incubated with naked telomerase RNA and RNase H (data not shown). In contrast, base pairing of both oligonucleotide 3 and oligonucleotide 8 with the telomerase RNA in the intact RNP can direct cleavage by RNase H. Cleavage by RNase H can occur with as few as six base pairs of DNA-RNA duplex[44]. Thus, the base-pairing interaction of oligonucleotides 3 and 8 with the telomerase RNP must be distinct from the way in which $d(TTGGGG)_4$ interacts with the RNP during the preincubation. This difference could be due to an alteration in the RNP structure induced by oligonucleotides 3 and 8, or it may reflect a real distinction between telomerase recognition and elongation. The initial recognition of telomeric primers may not involve the CAACCCCAA sequence of the RNA. This is consistent with our earlier observations that G-rich oligonucleotides with differing primary sequences corresponding to the telomeric sequence of a number of species, as well as synthetic telomere-like sequences, all efficiently prime repeat addition by telomerase[5,45]. Together, these data indicate that a primer can be delivered to the active site in two ways; telomeric G-rich sequence oligonucleotides are specifically recognized, possibly by their unusual secondary structural features[46], whereas some oligonucleotides complementary to the 159 RNA (like 3 and 8) can be positioned in the active site by base hybridization.

The elongation translocation mechanism. When oligonucleotide 8 was preincubated with telomerase and $d(TTGGGG)_4$ was added at time zero, the oligonucleotide 8-primed pattern was seen in the products, indicating that the elongation reaction is processive. This, together with the evidence that hundreds of repeats of TTGGGG are synthesized on the input primer, and that there are one and a half repeats of the template sequence, indicate that an elongation-translocation mechanism operates to synthesize many tandem repeats. Previous work showed that the sequence at the 3′ end of a telomeric primer is recognized by telomerase in the elongation reaction. A primer ending in TTGGGG 3′ is distinguished from those ending in TTGGG 3′, GGGGTT 3′ or TTGG 3′[5,45] and each primer is correctly filled out to complete the last TTGGGG sequence before further repeats are added. These data could be explained by the mechanism outlined in Fig. 6. After primer recognition, the most 3′ nucleotides of the primer are hybridized to the 3′ portion of the CAACCCCAA sequence and the primer is filled out to complete the last full hexanucleotide repeat. Translocation then repositions the primer so that its most 3′ nucleotides are again hybridized ready for elongation.

Such an elongation-translocation mechanism could contribute to the strong six-base periodicity of the *in vitro* addition reaction. Earlier work showed that varying the relative concentrations of dTTP and dGTP in the reaction could change the intensity of the bands in the *in vitro* synthesized banding pattern[4]. This dependance on nucleotide precursor concentrations is also seen for the *Oxytricha* and *Euplotes* enzymes (ref. 23 and D. Shippen-Lentz and E. Blackburn, submitted). Pausing upon the addition of dTTP, as well as primer translocation, may contribute to the six base periodicity in the *in vitro* reaction products. Depending on the nucleotide concentration used, either two or three strong stops are seen in the elongation pattern using $(TTGGGG)_4$ as

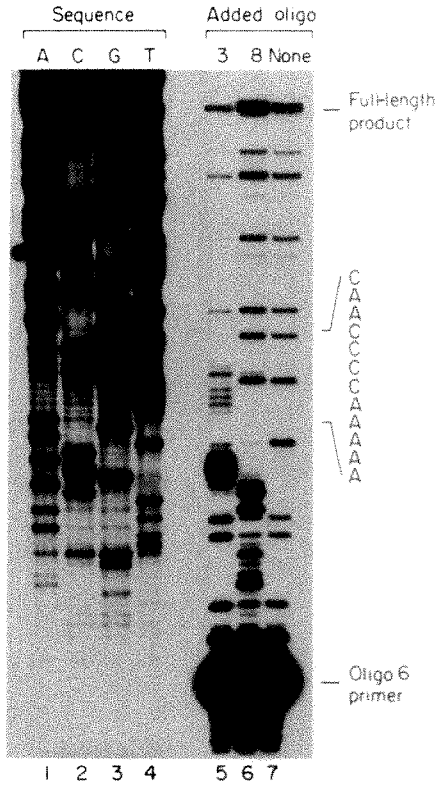


Fig. 5 Primer extension analysis of telomerase RNA cut with RNase H. Lanes 1–4, the sequencing ladder marker using oligonucleotide 6 as a primer. Lanes 5–7, the extension products from [^{32}P] oligonucleotide 6 on RNA extracted from telomerase fractions after treatment with RNase H and the oligonucleotides indicated above the lanes. The bands which appear in all of the lanes including lane 7, where no oligo was added, were probably due to the secondary structure of the 159 RNA. The full-length primer extension product is seen in all of the lanes because the cutting by RNase H in this experiment was not complete. The region corresponding to the CAACCCCAA sequence is indicated.

Methods. 400 μl of telomerase purified over a sizing column and heparin agarose, were incubated with 10 μl of the indicated oligonucleotide and 10 μl of RNase H (2U μl^{-1}) at room temperature for 30 min. 40 μl of a 10% SDS +100 mM EDTA solution and 10 μg of proteinase K were added. The mix was incubated at room temperature for 20 min and then the RNA was phenol extracted and ethanol precipitated after the addition of 5 μg of carrier tRNA. The pellet was resuspended in 6 μl of 125 mM KCl and 5 mM Tris *p*H 8.3. To 3 μl of RNA, 1 μl of [^{32}P] oligo 6 containing 0.05 μg was added and each mix was incubated at 65 °C for 5 min. The annealing reactions were then transferred to 42 °C for 1 hour. To each reaction 6 μl of elongation mix was added. The elongation mix contained 2 μl 5x reverse transcriptase buffer, 1 μl each dNTP at 2.5 mM, 1 μl avian myeloblastosis virus (AMV) reverse transcriptase (Life Sciences), 2 μl DEP-water. The elongation was carried out at 52 °C for 30 min. Sequencing loading dye (4 μl) was then added and 4 μl of each sample was loaded onto a 6% polyacrylamide sequencing gel. The sequencing markers were produced using oligonucleotide 6 as a primer following the procedure for supercoiled DNA sequencing using the Sequenase kit (USB).

the primer. The two strong stops correspond to the two T residues in the repeat[4] whereas the three stops correspond to the addition of the nucleotides TTG (data not shown). This pausing pattern is consistent with the model presented in Fig. 6 for elongation and translocation.

Implications of the internal RNA template. The specification of TTGGGG repeat addition by an internal template is analogous to the utilization of the internal binding site as a template in the reactions catalysed by the *Tetrahymena* intron ribozyme. This ribozyme has been shown to catalyse self splicing, *trans*-splicing, RNA endonuclease and RNA polymerase reactions[47–52]. In all of these reactions a sequence internal to the

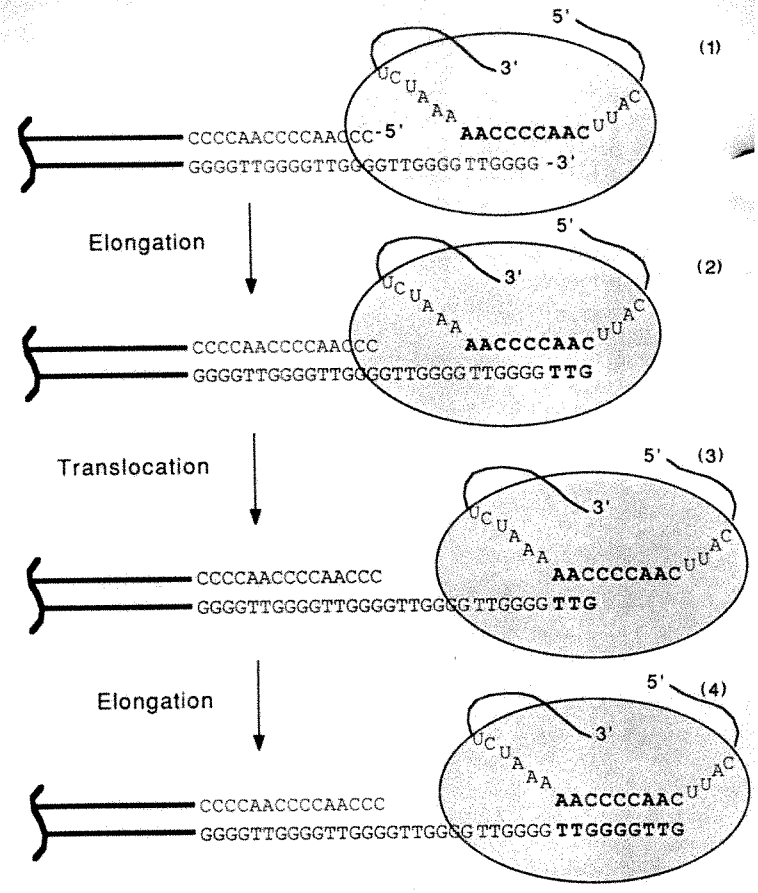


Fig. 6 Model for elongation of telomeres by telomerase. The *Tetrahymena* telomere is shown containing a 13-base overhanging TTGGGG strand[54]. (1) After recognition of the TTGGGG strand by telomerase, the 3′ most nucleotides are hybridized to the CAACCCCAA sequence in the RNA. (2) The sequence TTG is then added one nucleotide at a time[4]. (3) Translocation then repositions the 3′ end of the TTGGGG strand such that the 3′ most TTG nucleotides are hybridized to the RNA component of telomerase. (4) Elongation occurs again, copying the template sequence to complete the TTGGGGTTG sequence. This mechanism explains how oligonucleotides with 3′ ends terminating at any nucleotide within the sequence TTGGGG are correctly elongated to yield perfect tandem repeats of $(TTGGGG)_n$ (ref. 5).

ribozyme interacts with a substrate through Watson–Crick base pairing. Mutations in this internal binding site alter the substrate specificity of the ribozyme[47]. By analogy, it is predicted that changing the sequence of the internal template of telomerase would result in synthesis of telomeric repeats of that altered sequence. Whether the analogy between the telomerase and the intron ribozyme of *Tetrahymena* extends to include catalysis by RNA alone is a question which we can now begin to address.

DNA replication and RNA transcription represent conventional nucleic acid synthesis reactions directed by an external template. Terminal deoxynucleotidyl transferase, poly(A) polymerase and tRNA nucleotidyl transferase represent completely template-independent mechanisms of nucleic acid synthesis. In contrast to both of these types of polymerases, the *Tetrahymena* intron ribozyme and telomerase represent a new class of polymerase which is characterized by containing a template sequence that is an integral component of the polymerase itself. Although many RNPs, as well as the *Tetrahymena* ribozyme, use base pairing of their RNA components with other RNAs to determine reaction specificity[24–28,53], the telomerase enzyme is unique among eukaryotic RNPs in that it uses an RNA template for DNA synthesis.

The synthesis of DNA using an RNA template may be a very ancient process. Telomerase enzymes with RNA components may be relics that have descended from an ancestral type of DNA polymerase whose action was based on an internal RNA template. In this model, the primitive DNA polymerase was replaced in evolution by the current protein-based DNA polymerases and reverse transcriptases which use exogenous templates, whereas telomerase retained the endogenous template and was used by eukaryotes for telomere maintenance.

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LETTERS TO NATURE

Is N44C a fossil X-ray ionized nebula?

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Long-slit spectroscopic CCD observations of the H II region N44C (refs 1–3) in the Large Magellanic Cloud and its central hot, massive star[2,3] show that the stellar temperature is far too low to account for the high excitation of the nebula. In particular the strong nebular He II 4686-Å recombination radiation, which is absent in normal H II regions, cannot be understood in terms of ionization by the stellar ultraviolet flux. Here we propose an alternative interpretation in terms of a (fossil) X-ray photo-ionized nebula resembling the recently discovered He III region around the luminous X-ray binary LMC X-1 (ref. 4). In this picture the presently extinct X-ray source that has been responsible for the high ionization in N44C could well be identical with the transient LMC X-5 (refs 5, 6). From photon-counting arguments and recombination timescales of the interstellar plasma we find that less than 100 years ago the X-ray luminosity should have been comparable with that of the most luminous X-ray binaries (a few $\times 10^{38}$ erg s^{-1}). This hypothesis can be tested by future observations.

Among the most conspicuous differences that distinguish emission line spectra of H II regions from those of the generally more highly photo-ionized planetary nebulae is the absence, in the former, of the He II 4,686-Å recombination line, because even the hottest known massive stars (O stars) radiate only negligible amounts of photons more energetic than 4 Rydbergs (54.4 eV), which are of sufficient energy to ionize helium completely[7,8] (see below). A notable exception is the nebula N44C (NGC1936) in the Large Magellanic Cloud (LMC), which was reported[1] to exhibit an unusually high-excitation planetary-nebula-type spectrum that includes strong He II 4,686-Å recombination radiation ($I_{4,686}/I_{H\beta} = 0.07$). Subsequently[2], this region was studied by narrow-band photographic imaging of Hα and [O III] 5,007-Å emission surrounding a central star, which was proposed as the cause of the ionization.

These investigations have not been followed up until recently, when the nebular He II 4,686-Å emission was rediscovered[3] and an effective stellar temperature of 70,000 K was derived from the nebular recombination-line flux and the optical brightness ($V = 14.6$; K. Reinsch, personal communication) of the central star (star 2), using the Zanstra method. Luminous stars with such high temperatures have so far not been detected (the earliest spectral types, O3, correspond to $T_{\rm eff} = 45{,}000$–$53{,}000$ K)[9,10], but they are nevertheless predicted to exist during late (helium-burning) phases in the evolution of very massive stars[11–14]. Because of strong mass loss in a stellar wind, however, the surfaces of stars initially more massive than ~40 $M_\odot$ are expected to become strongly depleted in hydrogen and should appear as hot Wolf–Rayet (WR) stars[11–13]. Whereas the issue of WR-star temperatures is a matter of current debate (see ref. 15 and references therein), we note that some WR stars indeed emit a luminous He^+ Lyman continuum capable of photo-ionizing large He III regions. Examples of such systems are the galactic nebula G2.4+1.4 around the WO1 star LSS4368 (ref. 32) and part of the nebula N76A in the Small Magellanic Cloud, which is ionized by the WN star AB7 (M.W.P. *et al.*, manuscript in preparation). Because of heavy contamination by the nebular emission lines of N44C, the previously reported[3] spectrum of star 2 does not provide good indications of photospheric temperature and hydrogen abundance; the lack of broad emission-line features, however, already excludes a WR-type nature. Here we report results that do not support the suggestion of a very high surface temperature, and we suggest an alternative explanation for the high degree of ionization in N44C in terms of a fossil X-ray ionized nebula.

During four consecutive nights in January 1987 we carried out long-slit spectroscopic observations of N44C and its central star using the B&C spectrograph on the ESO 3.6-m telescope with a 520×320-pixel (30 μm size) RCA CCD detector and a spectral full-width-at-half-maximum (FWHM) resolution of 5.5 Å (1.6 pixel). Several different slit orientations were chosen to cover as much of the emission-line region as possible. The intensities of several diagnostic nebular lines were extracted as a function of position along the slit, and background stellar and nebular continuum emission at nearby wavelengths were subtracted (see ref. 4 for method). In these intensity histograms stellar absorption/emission lines appear as corresponding features with a width determined by the seeing profile during the observations (1.5–2.5 arcsec FWHM). Figure 1 illustrates the strong concentration of the H I and He I nebular intensity and the increase in helium and oxygen ionization towards the central star 2. The mean extent of the He III region, as measured from the He II 4,686-Å recombination line, amounts to 18 arcsec, corresponding to 4.5 pc at the distance of the LMC, but this emission can be traced even to 35 arcsec (9 pc) in a radial filament southwards of the centre. Unresolved dips are clearly present in all H-Balmer-line and He I 4,471-Å intensity histograms (corresponding to different slit orientations) at the position of star 2 (Fig. 1*a* and *b*). The latter feature is ~5–10 times narrower than the broad 4,471-Å/Hβ minimum around star 2, reflecting the reduced He I emissivity (proportional to He^+ density) in the He III region. We are confident, therefore, that the O star exhibits photospheric He I absorption with an estimated equivalent width (EW) of 0.2–0.4 Å.

From the CCD images we have also extracted the optical spectrum of star 2 by careful subtraction of the nebular contribution. Intensities and line ratios change so rapidly across N44C (and from pixel to pixel along the slit) that the elimination of nebular emission is far from perfect, causing spurious emission and/or absorption lines of nebular origin (Fig. 2). Nevertheless, the photospheric Balmer series in absorption confirms the presence of hydrogen in the atmosphere of star 2. Moreover, background subtracted spectra, aimed at an optimal cancellation of nebular Balmer lines, show that the Hγ absorption EW (2.2 Å) is typical of early O-type stars[9,10] and therefore imply normal hydrogen abundance. This result argues against star 2 representing an advanced hot stage of massive stellar evolution, because hydrogen would then be already strongly depleted on the surface[11–13] unless a very metal-poor composition and small mass-loss rate are assumed[14]. From spectral classification and temperature calibration of O stars[10,16], the observed He I 4,471-Å/He II 4,540-Å EW ratio (0.2–0.5) implies a spectral type O4 to O6 and an effective temperature in the range of 40,000–50,000 K. That star 2 is probably a moderately early main-sequence O star with little indication of mass loss is also supported by the lack of the N V 4,604–4,620-Å, N IV 4,058-Å and N III 4,634–4,641-Å features that are seen in early Of stars[16]. The absence of stellar He II 4,686-Å emission indicates that the star is not a giant or supergiant.

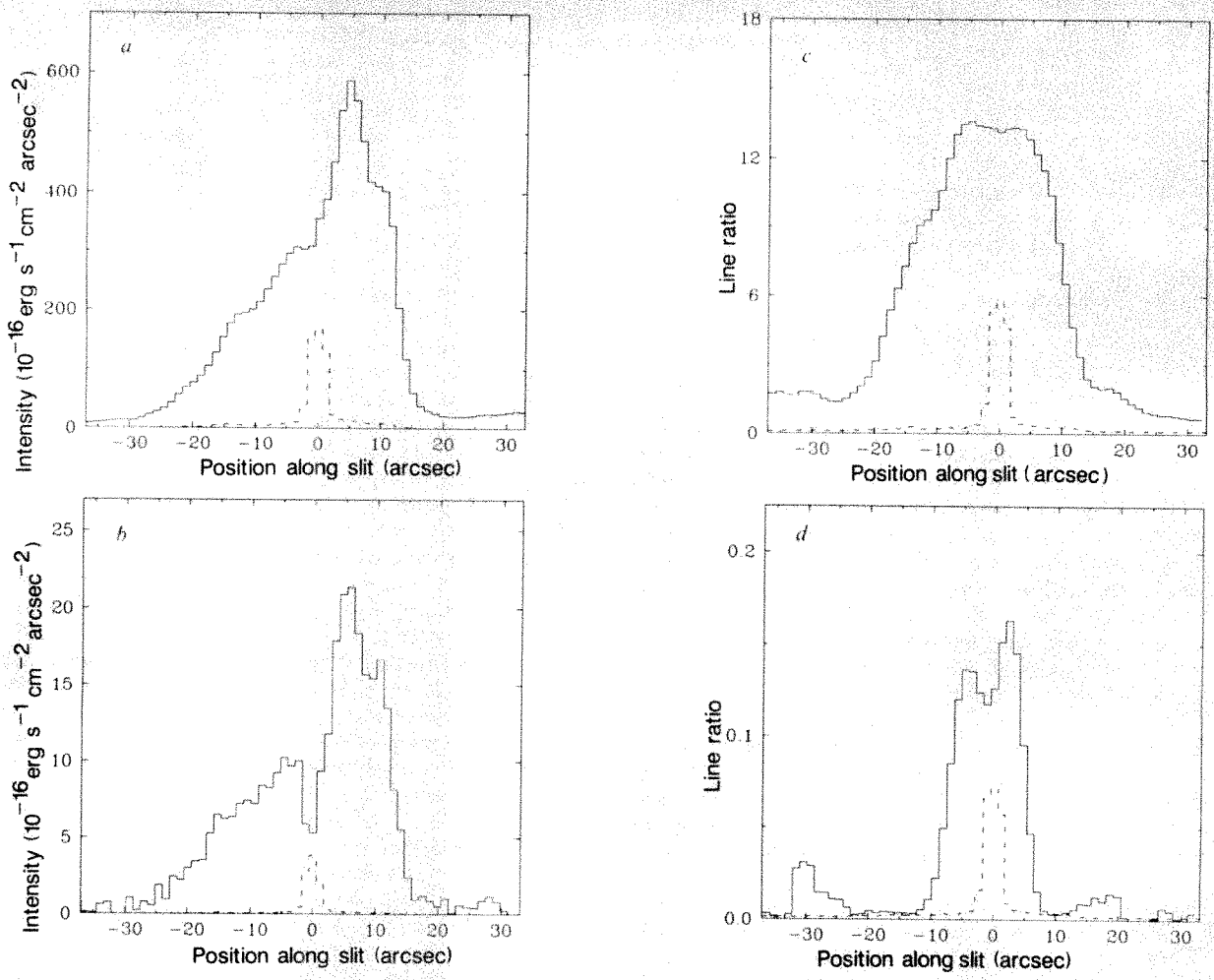


Fig. 1 Histograms of Hβ (*a*) and He I 4,471-Å (*b*) emission, and [O III] 4,959-5,007-Å/[O II] 3,727-Å (*c*) and He II 4,686-Å/Hβ (*d*) line ratios, in N44C as a function of position along the slit (position angle 313.°3; SE: left; NW: right). Stellar and nebular continuum flux at nearby wavelengths has been subtracted. The spatially unresolved dip in *b* indicates photospheric He I absorption in star 2; compare Fig. 2. The ionization structure in *c* and *d* is highly symmetric and is centred on star 2. The stellar continuum radiation scaled down by an arbitrary factor is shown for comparison in the dot-dashed curves. 1 arcsec corresponds to 0.25 pc in the LMC. The extent of the 4,686-Å emission (He III region) is 4.5 pc.

Recent calculations of nonlocal-thermodynamic-equilibrium-model atmospheres[17] of O stars indicate that the He^+ Lyman continuum can be strongly enhanced relative to plane-parallel models without stellar wind[7], when spherical extension and mass loss are taken into account. We have computed the expected 4,686-Å recombination flux, $F_{4,686}$, from such a model for the O4f star ζ Pup, to which star 2 is comparable except for its much weaker stellar wind. Using the well-known Zanstra method to link $F_{4,686}$ to the stellar brightness in the visual, we find that this model underestimates the 4,686-Å flux, measured as 1×

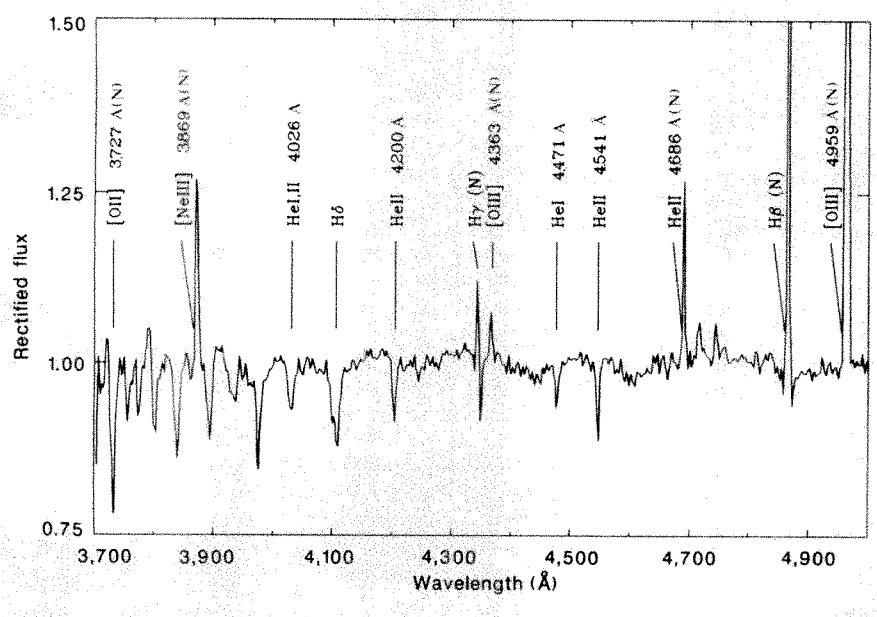


Fig. 2 Rectified optical spectrum of star 2. Spurious emission and absorption features (marked N) are due to imperfect subtraction of the nebular background. Note the presence of photospheric H I, He I and He II absorption lines, indicative of an O4–O6 V spectral type.

10^{-12} erg cm^{-2} s^{-1} (note that the value reported in ref. 3 is in error (G. Stasinska, personal communication)), by a factor of 130. Our observations therefore do not support the suggestion that star 2 is an extremely hot star capable of producing the N44C ionization structure.

An alternative interpretation[3], in terms of ionization by a strong stellar wind or supernova blast wave colliding with the interstellar medium, is also inconsistent with the observations. In this mechanism, high shock velocities in excess of 100 km s^{-1}, and accordingly high electron temperatures ($T_e(O^{2+})$ > 24,000 K), in the O III region are required to produce substantial fractions of He^{2+} ions[18], whereas the observed temperature[3] (12,000 K) clearly indicates that N44C is photo-ionized. We also note that the radial velocity of the 4,686-Å emission, 302 ±5 km s^{-1}, corresponds within 5 km s^{-1} to those of lower ionized species in the N44 complex, which effectively rules out a chance projection of a galactic planetary nebula onto the face of the LMC.

Guided by the strong resemblance of the optical emission-line spectrum to that of the He III region N159F surrounding the luminous X-ray binary LMC X-1 (ref. 4), we explored the conjecture that N44C represents an X-ray photo-ionized nebula (XIN). However, in the 0.15–4.5-keV Einstein satellite survey of the LMC (ref. 19), no X-ray emission from the vicinity of N44C was detected, and re-analysis of a previously unpublished Image Proportional Counter (IPC) observation of N44 (8,000 s integration time on 2 July 1980; F. Seward, personal communication) places an upper limit of 2×10^{35} erg s^{-1} on non-resolved X-ray emission from star 2. This value is 2,000 times smaller than the XIN hypothesis would predict (see below), excluding the possibility that the X-ray source was sufficiently active at that time. On the other hand, there is independent support for earlier X-ray activity in 1974–1976 from OSO-7 and Ariel V observations[5,6], which reveal a soft (2-keV) variable source, LMC X-5, at a celestial position consistent with that of N44C, although the relatively large errors preclude an unequivocal identification[19,20].

Adopting the fossil XIN interpretation of N44C, we can estimate the former luminosity of the currently inactive X-ray source. Drawing on photon-counting arguments[4] for the XIN around LMC X-1 and assuming the same soft X-ray and extreme ultraviolet spectral distribution for both sources, the observed flux $F_{4,686}$ corresponds to a recombination line luminosity, $L_{4,686}$ of 3×10^{35} erg s^{-1}, and therefore implies a 2–10-keV X-ray luminosity, L_x of $\sim(2\text{–}4)\times10^{38}$ erg s^{-1}. As some $He^{2+}\rightarrow He^{+}$ recombination must have occurred since X-ray activity ceased, this value represents a lower limit, implying that the source must have ranked amongst the most luminous X-ray binaries known.

A further important indicator of high nebular ionization is [Ne V] 3,426-Å emission, which line is produced at an ionization potential of 97.1 eV. This line is present in the XIN N159F (ref. 4) with a maximum intensity relative to Hβ, $I_{3,426}/I_\beta$, of 0.3, but is not detected in N44C with an upper limit of 0.05. How can this observation be reconciled with the fossil XIN hypothesis? To answer this question we note that recombination of Ne^{4+} is determined mainly by electron capture; charge-transfer reactions with H^0 are negligible because the ultraviolet radiation of star 2 maintains strong hydrogen ionization in the nebula. The recombination timescale is therefore $\tau_{Ne^{4+}} = (\alpha(Ne^{4+}, T)\, n_e)^{-1}$ where $\alpha(Ne^{4+}, T)$ ($=9\times10^{-12}$ cm^3 s^{-1} for $T = 10{,}000$ K (refs 21, 22)) is the Ne^{4+} recombination coefficient and n_e is the electron density. Therefore, [Ne V] 3,426-Å emission decays on a timescale of 16 $(n_e/200\ \text{cm}^{-3})^{-1}$ years after the extinction of the X-ray source (an electron density of 200 cm^{-3} has been assumed to be representative for N44C (ref. 3)). As recombination of He^{2+} takes place on a longer timescale ($\tau_{He^{2+}} = [\alpha(Ne^{4+}, T)/\alpha(He^{2+}, T)]\ \tau_{Ne^{4+}} = 6.5\ \tau_{Ne^{4+}}$), the combination of strong 4,686-Å and the absence of 3,426-Å radiation in N44C allows us to directly estimate the time, t_{off}, that has elapsed since the switch-off the X-ray source. Taking into account light travel-time effects, we find $20\ \text{yr} < t_{off}(n_e/200\ \text{cm}^{-3}) < 100$ yr. $\tau_{He^{2+}}$ also represents the timescale for the formation of the He III region[23], and therefore provides a lower limit, t_{on}, for the duration of strong X-ray activity in the past ($t_{on} > 10^2\ (n_e/200\ \text{cm}^{-3})^{-1}$ yr).

The concept of fossil nebulae being in an ionization state that is inconsistent with the presently observed ultraviolet sources they contain has been discussed previously in the context of planetary nebulae[24], H II regions, active galaxies (ref. 25 and references therein) and the interstellar medium around young supernova remnants[26,27]. However, the evolutionary timescales associated with significant decreases of the ionizing flux are in most cases much longer than the recombination and cooling timescales in the nebulae. The estimated lifetime of highly luminous massive X-ray binaries ($\sim10^4$–10^5 yr (ref. 28)) seems also to be too large to render fossil XINs observable. On the other hand, several 'permanent' sources (as opposed to the short-lived transients) are known to switch off for extended (>several years) time intervals[29,30], demonstrating that in these systems, X-ray active phases can alternate with periods of quiescence on timescales much shorter than those determined by stellar evolution. (Although this phenomenon has not been observed previously in close X-ray binaries with O-star primaries.) This as yet poorly understood behaviour implies the existence of a non-negligible number of fossil XINs which are observable if the density of the interstellar medium is sufficiently high.

A key test of the XIN hypothesis for N44C is the prediction that orbital motion of the advocated close compact binary companion causes star 2 to exhibit periodic radial-velocity and possibly ellipsoidal light variations. From our observations we derive an upper limit of 60 km s^{-1} to any such velocity variations. This value is of the same order as the radial-velocity amplitudes displayed by the O-star companions of several luminous X-ray pulsars[31] to which the proposed, presently inactive X-ray binary in N44C might be compared.

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Orbital evolution of circumplanetary dust by resonant charge variations

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Vast ethereal rings composed of micrometre-sized particles have been detected about the three giant planets so far visited by Voyager. Jupiter's diffuse halo, main ring[1] and gossamer ring[2] are visible because of the dust they contain. Small particles are also important constituents of Saturn's tenuous outlying rings: the contorted slender F ring[3], the G ring[4–6] and the extensive E ring[6,7]. At Uranus, dust was discerned by imaging[8] amidst, as well as inwards of, the nine previously known narrow rings; plasma measurements[9,10] suggest that micrometre grains are also present in an orbit of 4.4 times the planet's radius, where Voyager 2 pierced the planet's equatorial plane. Here we identify an orbital evolution mechanism for gravitationally dominated dust that may temporarily overwhelm other processes, and we derive evolution rates that agree with numerical integrations; we also indicate briefly how this process could affect the location of the faint rings evident today.

The orbits of micrometre-sized grains in planetary rings are governed mainly by gravity, but electromagnetic forces provide significant perturbations[11,12]. Such effects accumulate at discrete locations in the rings, Lorentz resonances[13], where specific components of the perturbations match orbital periods and cause orbital eccentricity e and inclination i to be large. These positions may determine the boundaries of Jupiter's ring[1,11,13]. But Lorentz resonances and electromagnetic processes in general have not previously been thought to change secularly the orbital size (semi-major axis a), although gyro-phase drift[14] and diffusive transport[15] may be effective for the tiniest grains (radius $s \lesssim 0.1\ \mu m$)[6, 12, 16, 17]. The orbital evolution of dust has been ascribed instead to plasma (Coulomb) drag, with the Poynting-Robertson effect being a lesser contributor[15,16,18].

Grains become electrically charged because any surface in space develops a potential through photo-ionization by solar radiation and through the currents carried by the local plasma to their surfaces. About the giant planets, photo-ionization can be ignored if the magnetospheric plasma is dense (number density $\eta \geq 10^2\ cm^{-3}$) and/or energetic. Thus, assuming perfect absorption of an isotropic and maxwellian plasma and no secondary emission, and ignoring the discrete nature of the charging process, the history of a particle's instantaneous charge Q is followed by summing the flows of positive and negative ions to the grain[19,20]:

$$\frac{dQ}{dt} = \pi s^2 \eta q w \left[\left(1 + \frac{\alpha^2}{2w^2} + \frac{2q|\Phi|}{m_i w^2} \right) \mathrm{erf}\left(\frac{w}{\alpha} \right) + \frac{\alpha}{w\sqrt{\pi}} e^{-(w/\alpha)^2} \right] - 2\sqrt{\frac{2\pi kT}{m_e}} s^2 \eta q e^{q\Phi/kT} \quad (1)$$

where q and m_e are the electron charge and mass, w is the speed of the grain relative to the mean plasma (assumed co-rotating with the planet), T is the plasma temperature, $\alpha = \sqrt{2kT/m_i}$ is the thermal speed of the ions, and k is the Boltzmann constant. The grain's potential $\Phi = Q/s$ reaches equilibrium when $dQ/dt = 0$. The equilibrium potential $\bar{\Phi}(T, w) = \bar{Q}/s$ is shown in Fig. 1 for plasma conditions that might apply to a jovian ring grain. The dependence of Φ on w principally reflects changes in the ion flux to the grain, because the electron flux is essentially independent of w when $\sqrt{2kT/m_e} \gg w$. A stationary grain develops a negative charge to ward off the swift electrons. For $w/\alpha \lesssim 2$, the ion current is reduced (because Coulomb attraction is less effective at focusing ions) so that $\bar{\Phi}$ decreases further, and eventually, at large w/α, the ion current is due primarily to ram charging onto the grain, so that $\bar{\Phi}$ increases linearly with w/α.

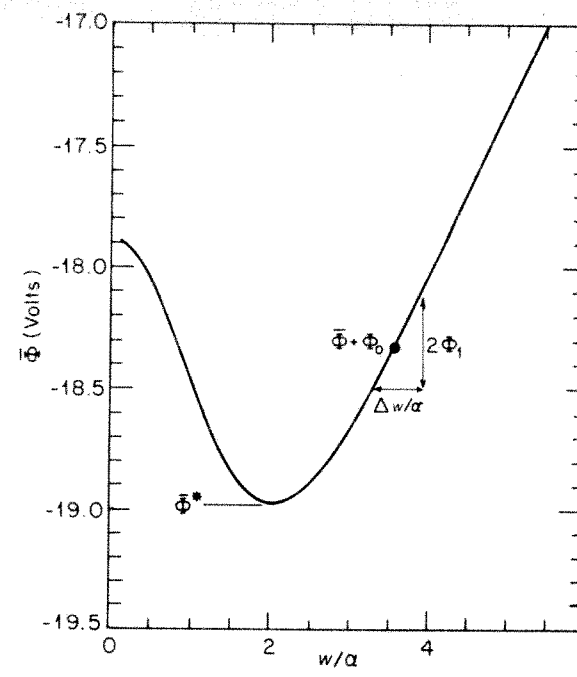


Fig. 1 A grain's equilibrium potential as a function of the ratio of w, the grain's speed through the plasma, to the ion thermal speed α for a plasma of O^+ at a temperature of 5 eV. Φ^* is the minimum potential that can be attained. Φ_1 is roughly the variation in the potential that occurs as w/α changes with high η.

Q will vary systematically during the grain's orbit because the state of the plasma (η, T and even composition) varies with position and because a grain's velocity relative to the ambient co-rotating plasma varies along an elliptic orbit. Expanding equation (1) about $\bar{Q}$ (ref. 21) gives an inhomogeneous linear first-order differential equation for $\Delta Q = Q - \bar{Q}$ showing that small harmonic changes in w, η or T cause harmonic variations in ΔQ, which are phase-delayed because it takes some time to acquire the charge appropriate to local conditions. Allowing for large orbital excursions, the grain's charge can be expressed in general (compare refs 17 and 21) as

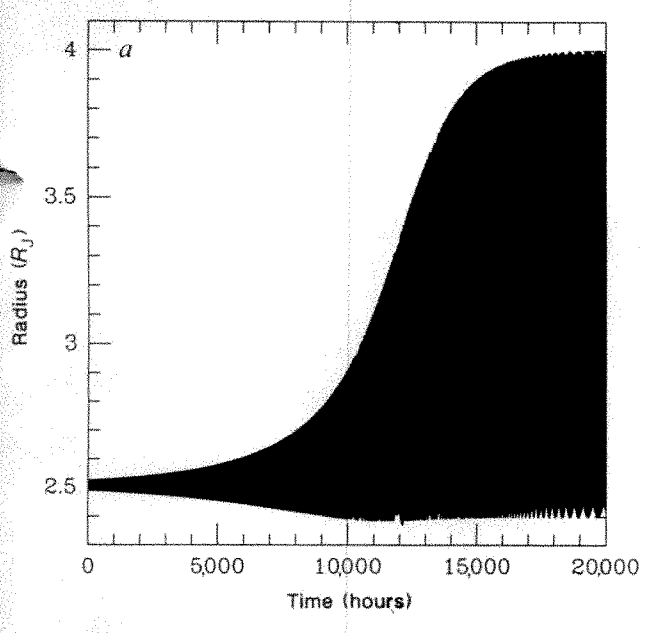
$$Q = \bar{Q} + Q_0 + Q_1 \cos(\theta - \theta_1) + Q_2 \cos 2(\theta - \theta_2) + \ldots \quad (2)$$

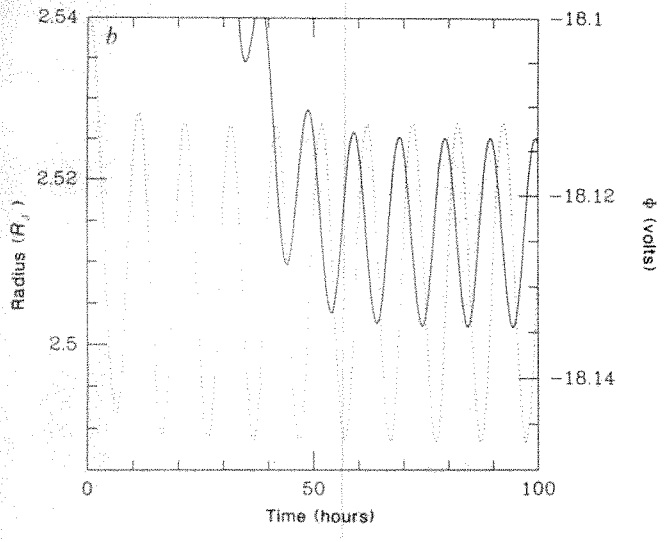
where time has been replaced by θ, the orbit's true anomaly or its angular position from pericentre, and where (Q_i, θ_i) are the strengths and phase lags of the various Fourier terms, which will be functions of the mean orbital position.

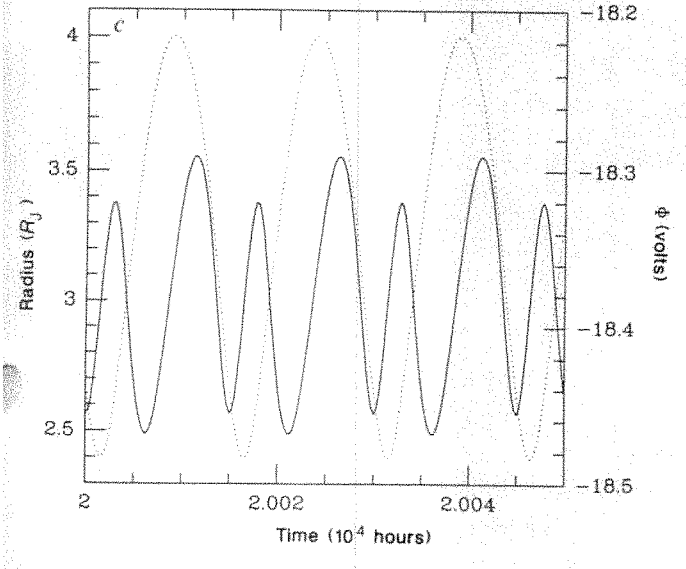
Present evidence indicates that particle sizes and potentials in tenuous planetary rings are such that motions are gravitationally dominated[6,11]. Here we concentrate on the orbital evolution caused by the charge variations produced, in turn, by velocity changes along an elliptical orbit. The case of electromagnetically dominated grains, where particles gyrate about and drift across magnetic field lines, has been analysed previously[14] and applied to a jovian grain's evolution[17].

Figure 1 indicates the charge variation along an orbit if the charging time is much less than an orbital period; $\bar{Q} + Q_0$ is then the mean charge for a particular orbit and Q_1 is roughly the maximum excursion about this value; Q_2 and higher-order

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terms are present only when large changes occur in w/a (so that linearization in velocity variation is inappropriate) or when the orbit is such that the minimum potential $\bar{\Phi}^*$ (Fig. 1) is achieved. The Q_1 term indicates that a part of the charge varies with the orbital period; hence a delayed resonant perturbation acts on the particle, causing orbital evolution by a process that we call resonant charge variation.

We consider grains moving through the equatorial plane of an axially symmetric magnetic field:

$$\mathbf{B} = B_0(R/r)^3[1+\beta(R/r)^3+\ldots]\hat{\boldsymbol{\phi}}$$

where B_0 is the dipolar magnetic field at the planet's surface ($r = R$), $\beta(\ll 1)$ is the relative octopole strength and $\hat{\boldsymbol{\phi}}$ is the unit normal to the equatorial plane in the direction of the planet's spin $\boldsymbol{\Omega}$. Near the planet, the plasma is carried by the magnetic field, producing, in the inertial reference frame, a 'co-rotational' electric field $\boldsymbol{\mathcal{E}} = \Omega R b\hat{\mathbf{r}}/c$, where c is the speed of light and $\hat{\mathbf{r}}$ is the radial unit vector.

The orbital evolution of semi-major axis a and orbital eccentricity e is determined solely by the rates of change of the orbit's specific mechanical energy, $E = -GM/2a$, and angular momentum, $\mathbf{H} = \sqrt{GMa(1-e^2)}\hat{\boldsymbol{\phi}}$ (ref. 22); G is Newton's gravitational constant and M is the planet's mass. Thus, $a^{-1}\,\mathrm{d}a/\mathrm{d}t = \dot{E}/E$ and $\mathrm{d}e^2/\mathrm{d}t = (e^2-1)(2\dot{H}/H + \dot{E}/E)$. To integrate $\dot{a}/a$ over an orbit in the inertial frame, only the work done against $\boldsymbol{\mathcal{E}}$ needs to be considered because magnetic forces do no work ($\mathbf{v}\cdot(\mathbf{v}\times\mathbf{B}) \equiv 0$). Thus the semi-major axis changes at the average rate

$$\frac{1}{a}\frac{\Delta a}{\Delta t} = \frac{2a}{mGMP_0}\oint Q\boldsymbol{\mathcal{E}}\cdot\mathrm{d}\mathbf{r} \qquad (3)$$

where m is the grain's mass and the path integral is taken over a complete orbital period P_0. Using the magnetic-field expression above, we find

$$\frac{1}{a}\frac{\Delta a}{\Delta t} = \frac{B_0\Omega(R/a)^3 e}{nmc(1-e^2)}\left\{Q_1\sin\theta_1 + \frac{\beta(R/a)^2}{(1-e^2)^2}\times [Q_1(1+e^2/4)\sin\theta_1 + Q_2\sin 2\theta_2] + \ldots\right\} \qquad (4)$$

where $n = 2\pi/P_0 = (GM/a^3)^{1/2}$ is the orbital mean motion.

Similarly,

$$\Delta\mathbf{H} = \frac{B_0R^3}{mcH}\oint Q\dot{r}\,\mathrm{d}\theta\hat{\boldsymbol{\phi}} \qquad (5)$$

The co-rotational electric field does not enter because, being radial, it produces no torque. From this, the rate of change of e can be calculated; it is written most compactly as

$$\frac{\Delta e^2}{\Delta a/a} = (1-e^2) - (1-e^2)^{1/2}\left(\frac{n}{\Omega}\right) \qquad (6)$$

which can also be derived from the problem's Jacobi integral[21].

We can compare results from these analytical expressions with numerical simulations of a charged grain's evolution. We use a Runge–Kutta scheme[11,21] that, given a grain's position and velocity, computes accelerations resulting from Lorentz

◀ **Fig. 2** *a*, The orbital history of a 1-μm grain of density 1 g cm^{-3} placed in a 5-eV O^+ plasma ($\eta = 1$ cm^{-3}). The grain was launched on an initially circular equatorial orbit at 2.54 R_J, R_J being the radius of Jupiter. The parameters are chosen to produce rapid evolution. *b*, The grain's orbital radius r (dotted) and its electric potential Φ (solid) are shown with finer time resolution during the early evolutionary portion of Fig. 2*a*. The orbital period is roughly 10 hours. The phase lag θ_1 is evident. *c*, The same quantities as in *b* but plotted at a later time, when the charge variation is primarily doubly periodic and the orbit has stabilized. Because of the greater distance to Jupiter and the larger Lorentz force, the period has lengthened to almost 15 hours.

forces plus non-point-mass gravity and, through Newton's equations, the consequent orbital evolution; simultaneously, the grain's charge state is followed by using equation (1). Figure 2 shows the numerically determined orbital history of a jovian dust grain, using the appropriate values for Jupiter, $B_0 = -4.092$ G and $\beta = 0.028$ (compare ref. 11). The orbit quickly becomes eccentric because the initial path is not in centripetal equilibrium once the change develops, which occurs rapidly. As deduced from Fig. 1 and equation (2), charge varies at first primarily with the orbital period, like w, and exhibits a phase lag θ_1.

After ~9,000 hours, the grain evolves rapidly outward while its eccentricity grows. This drift can be understood from equations (3) or (4): because of the phase lag, the grain's mean charge is slightly more negative during its inbound motion than on its outbound path (see Fig. 3). The electric field thus does positive work during each radial oscillation and the orbit size expands. When e has reached a value of ~0.2, the evolution slows dramatically, for two reasons. First, as Fig. 2c suggests and a Fourier transform attests, Q then varies primarily in the second harmonic, which, for the same charge variation, is 10^{-2}–10^{-3} times as effective as Q_1, according to equation (4). Q_2 has developed because, with such a large e, w/α ranges from about 0.2 to nearly 4 (see Fig. 1); in addition, θ becomes a multi-periodic function of time, inducing Q_2. Second, despite the large excursion in w/α, charge variations (especially Q_1) are fairly small because the changes in w/α occur too swiftly for equation (1) to respond. The numerically computed rates, based on measured Q_i and θ_i, compare very favourably with our analytical expressions. We note that the very low η, chosen here to cause a large θ and a rapid evolution, is incompatible with our neglect of photo-ionization; under these circumstances, resonant pulsations in Q caused by passage through the shadow are important perturbations (M. Horanyi, personal communication).

In many of our numerical experiments the orbit size changes faster than would be expected from either plasma drag[6,15,16] or Poynting-Robertson (P-R) drag[23]. For example, during the steep-sloped mid-portion of Fig. 2a, $\dot{a}$ is about 250 cm s^{-1}, whereas the plasma drag and P-R evolution rates are two to three orders of magnitude less[6,17]; in denser plasmas, Coulomb drag could, however, dominate orbital evolution because its strength would increase at the larger density whereas the evolution caused by resonant charge variation would decrease. Nevertheless, the orbit's stabilization, which is apparent in Fig. 2a and which, as we have argued, is implicit in the charging scheme when velocity variations drive equation (1), means that dust will not inevitably leave the system.

The orbital evolution (equations (4) and (6)) will depend upon the nature of the charge variation ($Q_1, Q_2, \ldots, \theta_1, \theta_2, \ldots$), which in turn will be fixed by the orbit, because the latter imposes the time history for w, T and η in the charging relation (equation (1)). Other orbits or different plasma environments can produce

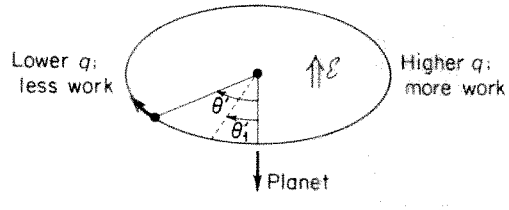


Fig. 3 The motion of a charged, orbiting grain as seen in a reference frame rotating at the mean orbital rate. The particle moves clockwise along this curve from pericentre (nearest the planet) to apocentre and back to pericentre. Because of the phase lag θ'_1, the mean charge on the right-hand side of the path is more negative than on the left-hand side so that more work is done moving toward the planet than away from it. A similar argument shows that the second harmonic is much less efficient in moving grains (compare equation (4)). For small eccentricity the plotted angle θ' approximately equals the true anomaly θ.

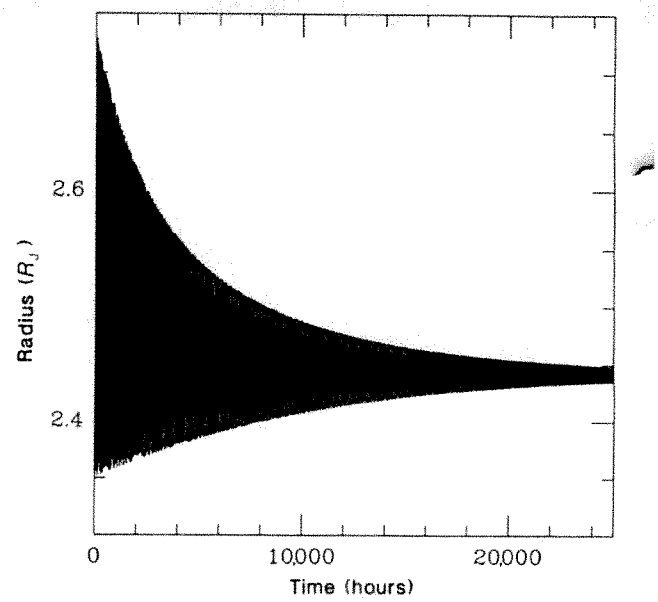


Fig. 4 The orbital history of a 0.34-μm grain of density 1 g cm^{-3} placed in a 0.2-eV O^+ plasma with density $\eta = 10$ cm^{-3}. The particle is introduced with some initial radial velocity to provide enough eccentricity to initiate noticeable evolution.

dramatically distinct histories. Figure 4 shows a grain placed in the same position as for Fig. 2 but in a plasma such that the particle's potential is to the right of $\bar{\Phi}^*$ on Fig. 1, and changes more quickly. A smaller grain size is chosen to make the perturbation forces comparable in the two examples. The grain's evolution is opposite, and the particle moves slowly toward the synchronous position while the orbit circularizes.

What might orbital evolution by resonant charge variation imply for the genesis of the faint rings? Because dust from a single source can be disseminated widely to populate a region both inside and outside the source (compare Fig. 2), it is possible for Enceladus to supply the whole E ring of Saturn, which straddles the satellite's orbit and extends out as far as Rhea's orbit. Similarly, the faint material surrounding Saturn's F ring[6,24] may be the evolved debris from collisions occurring in the ring itself. Given appropriate plasma conditions in the jovian magnetosphere, grains from distributed parents in the gossamer ring could move toward synchronous orbit to produce the enhancement observed there (Fig. 4; compare refs 2 and 17); this would require either that w/α be more than several (which is difficult to achieve near synchronous orbit) or that T increase toward synchronous orbit. For Uranus, the dust detected at $4.4R_U$ may have drifted inward from the outlying classical satellites rather than, as previously maintained[10], necessarily being derived from the small moon 1985U1, the only known object between synchronous orbit and the point of detection.

Resonant charge variations can produce rapid orbital evolution of dust, but the precise rate, and even the direction, of evolution depends sensitively on plasma parameters and dust sizes in inner planetary magnetospheres. Because these quantities are not as yet well determined, it is very difficult to predict the transport of dust within faint planetary rings.

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The winter solstice phenomenon at Newgrange, Ireland: accident or design?

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On Midwinter's Day, around four and a half minutes after sunrise, the sun shines down the 'roof-box' of the Neolithic passage graves at Newgrange, Ireland, and illuminates the floor of its main chamber 18 m away. When the monument was constructed, however, first light would have occurred at sunrise in the form of a very narrow beam bisecting the chamber. Here I suggest that the width and height of the gap in the floor of the roof-box may have been deliberate, tracing the path of the Sun at the solstice. Newgrange predates the astronomical structures of Stonehenge by 1,000 years and as such may be the oldest astronomically orientated structure in the world.

Located some 50 km from Dublin, Newgrange is the largest in a group of Neolithic passage graves in the Boyne Valley, County Meath. Although discovered in 1699, the tomb was not properly excavated until the 1960s[1]. It consists of a passage and chamber off which are three smaller alcoves (Fig. 1), giving the structure an overall cruciform appearance. Surrounding it is a large circular mound, over 80 m in diameter, of loose stones, and encircling this again is an incomplete ring of standing stones. The mound and ring are, however, not concentric and probably not contemporaneous.

As early as 1909, Lockyer[2] remarked that the passage grave was approximately aligned to the rising Sun at midwinter. He did not, however, pay much attention to the site. During recent excavations of Newgrange, O'Kelly[1] unearthed a curious decorated structure located above the entrance to the tomb. This structure, which was later to be called the 'roof-box'[1], covered a vertical gap between the first and second roof slabs of the passage. Conscious of a local tradition that the chamber was illuminated by the Sun at a certain time of year, O'Kelly[1] investigated whether the roof-box had some solar function, and found that the midwinter Sun shone through the roof-box and gap to illuminate the central chamber of the tomb (Fig. 2) 18 m away. This event would have occurred even if access to the tomb was blocked by the entrance stone[1].

Patrick[3] later concluded that the orientation of the roof-box towards the winter solstice was deliberate. Heggie[4] has pointed out, however, that on the basis of Patrick's calculations, the Sun would illuminate the main chamber if its declination lay at any position between −22°58′ and −25°53′, and hence the probability of a solsticial alignment in the corresponding range of azimuths is about 1 in 13. Heggie[4] concluded that this fact was 'not really significant enough to excite much interest'. But Heggie's calculation is in error for two reasons. First, as will be discussed below, Patrick's upper azimuth limit is about 1° too high and hence the declination range should be reduced accordingly. Second, and more importantly, the three-dimensional aspect of this problem has been ignored. As can be seen from Fig. 2, the solsticial light from the roof-box meets the floor at a glancing angle within the main chamber. If the gap between the first two roof-slabs was, say, 20 cm lower, or equivalently the passage was a few metres longer, sunlight would not enter the chamber. In fact, one would then observe the Boyne Valley, rather than the local horizon, through the gap. Correspondingly, if the gap was higher, the solsticial light would have been projected onto the back wall. Although such an alignment is of course admissible for statistical purposes, it would not have as dramatic an effect as that of a glancing beam on the floor. Thus the chance of Newgrange being accidentally aligned with a solsticial sunrise/sunset point is smaller, at least by a factor of two, than the figure quoted by Heggie[4]. Nevertheless the evidence still remains weak and it would not rival that found for sites such as Stonehenge[4] and Kintraw[5]. To test the solar hypothesis further, we re-surveyed the roof-box, as seen from the main chamber, and the local horizon in the direction of sunrise on Midwinter's Day.

The passage itself is sinuous (Fig. 1), restricting the range of azimuths from which a ray of light can enter the main chamber. We found this range to be 133°49′-137°29′ at the chamber

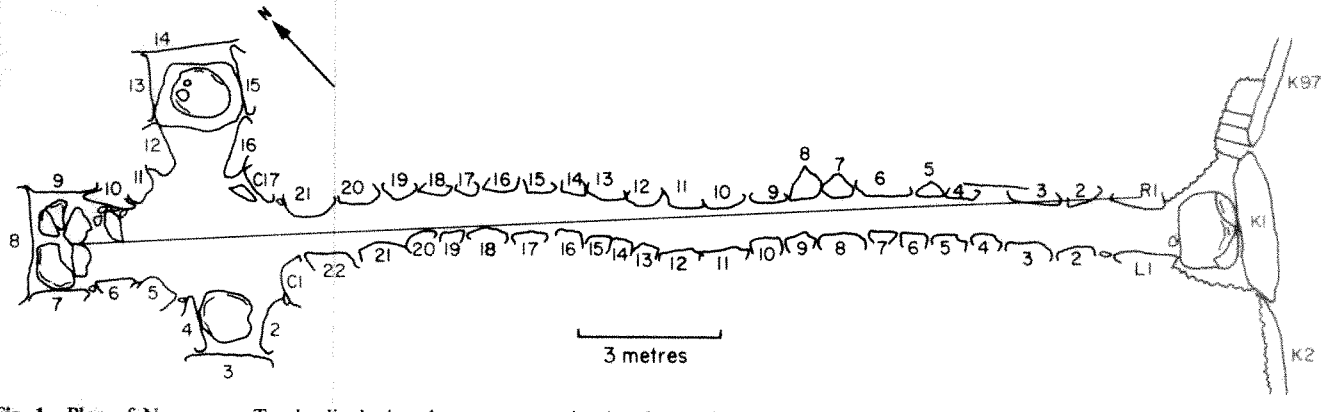


Fig. 1 Plan of Newgrange Tomb, displaying the passage, main chamber and its three alcoves. The marked line shows the minimum azimuth or 'first-light' axis of the passage. This axis coincides with the direction of Midwinter sunrise 5,150 years ago, when the tomb was built. Adapted with permission of C. O'Kelly.

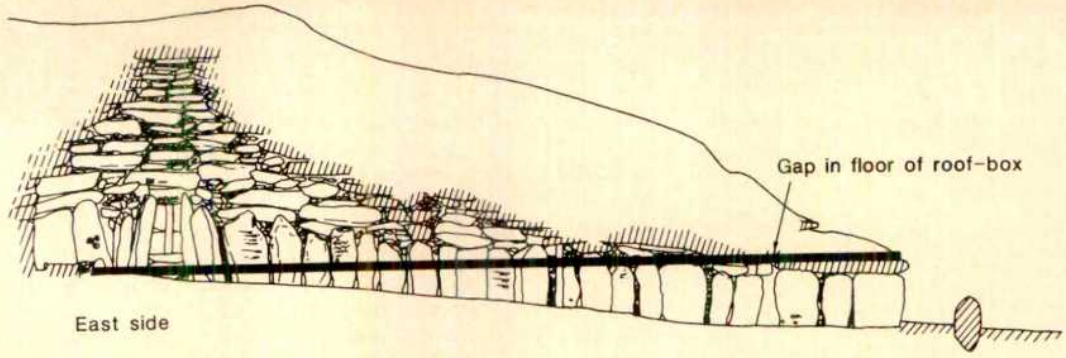


Fig. 2 Sectional elevation of the east side of Newgrange. The path of the first rays of the Sun around 3,150 BC is indicated.

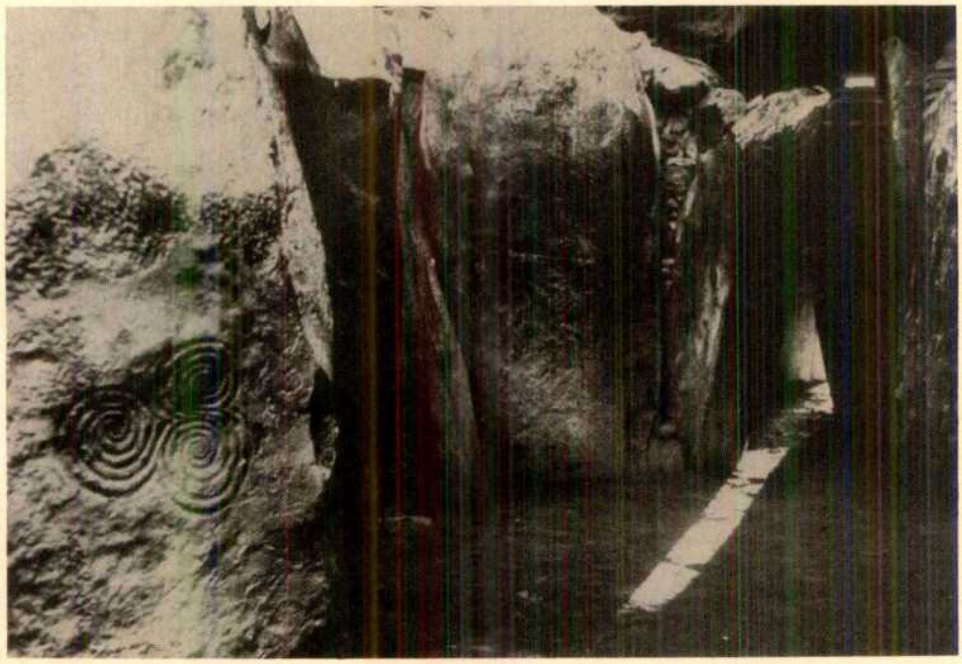


Fig. 3 Sunlight in the main chamber at the time of the winter solstice. The three-leafed spiral figure can be seen in the backmost alcove. Photo courtesy of Conn Brogan, Office of Public Works, Dublin.

entrance, with a typical error of about ±3′. The upper limit is ~1° greater than that quoted by Patrick[3]. We measured the light beam to be just under 34 cm wide near the entrance although it narrows towards the back recess, largely because stone L20 leans inwards. Close to the entrance to the chamber, however, L20 restricts the width of the light beam by 8 cm at most, so that if L20 were uprighted the minimum azimuth would be less than the quoted value by only 20′. The chamber floor is slightly lower than the gap in the roof-box (Fig. 2) and hence at low altitudes, the light of the Sun is spread across the floor. The minimum altitude at which sunlight can enter the chamber is given by the height of the local horizon, 55′ ± 2′, whereas the maximum altitude, confirmed by actual timing measurements of when the Sun disappeared (F. Prendergast, private communication) from the main chamber, is 2°11′ ± 2′. Using height measurements of the gap behind the roof-box and the chamber floor, we found that light at minimum elevation would reach at least 1 m into the back recess (Fig. 1). However, sunlight is now seen to extend only as far as the edge of this alcove, because the minimum azimuth axis, or 'first light' axis, of the passage does not point towards midwinter sunrise. In fact, light from the right-hand limb of the Sun first enters the main chamber four and a half minutes after sunrise (F. Prendergast, personal communication), and at that time the Sun is almost completely above the horizon.

The question then arises as to what would have been the solar alignment when the monument was built. Carbon dating of two charcoal samples from between the roof-slabs yielded radiocarbon ages of 4,450 ± 40 and 4,460 ± 45 BP[1]. To convert this to a calendar date, we have used the dendrochronological record provided by samples of Irish Oak[6]. This indicates that the probable construction period for the site is 3,150 ± 100 BC. For these dates the obliquity of the ecliptic would have been[7] 24°2′ ± 1′ and the midwinter Sun would rise at an azimuth of 133°54′ ± 4′. Here we have assumed, for calculating refraction[8], a pressure of 1,010 mbar, a temperature of 0 °C and the height of the local horizon to be 55′ ± 2′. Variations in temperature and pressure make very little difference to the result. We see that the Sun would have risen just within the limits of the roof-box as seen from the main chamber. In other words, the 'first-light' axis of the passage corresponds, within 5′ or so, to midwinter sunrise 5,150 years ago. If, at that time, one assumes that stone L20 was in an upright position, the accuracy would still be better than 25′. It follows that when Newgrange was built, the first beam of sunlight on the floor was less than 10 cm wide, approximately 2 m long, and bisected the chamber (Fig. 1). Rather curiously, this beam would have entered the back recess and indirectly illuminated a three-leafed spiral figure on its wall (see Fig. 3), as mentioned in legend[3,4,9].

As the Sun's altitude and azimuth increased, the beam would have become gradually broader. The height of the gap behind the roof-box varies between 18 and 22 cm, being 20 cm on average. As seen from the main chamber 18 m away, this gap subtends an angle of roughly 38′, so that the risen Sun is framed

in the vertical direction. Finally, the lower edge of the Sun would have left the roof-box, as seen from the main chamber, at an azimuth of 137°42′ ± 5′. Again this is very close to the maximum azimuth of the passage, implying a symmetry with respect to the Sun's passage at the winter solstice. Thus not only the height but also the width of the gap may be significant.

Newgrange predates the astronomically orientated structures (phase III) of Stonehenge by about 1,000 years. The evidence presented here supports the theory that the orientation of Newgrange was deliberate, which would make it therefore the oldest megalithic structure known for certain to have an astronomical function[4,5]. The alignment at Newgrange was within a fraction (that is, within $\frac{1}{2}°$) of the Sun's disk although it is doubtful whether the accuracy extends to the level of a few arcminutes. Newgrange should be seen in the light of large-scale studies such as those of Ruggles[10], which show definitive evidence that megalithic man was interested in marking the southern limits in declination of the Sun and Moon, albeit approximately. Such low accuracies suggest that ancient man's interest in these bodies may have been ritualistic rather than for the purpose of calendar construction.

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A superconducting copper oxide compound with electrons as the charge carriers

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Since the discovery of high-temperature superconductivity in copper oxide compounds[1], there has been much effort to understand what is required for a compound to have a high transition temperature (T_c). Up to now, all of the families of high-T_c copper oxide superconductors have contained two-dimensional sheets of Cu–O pyramids or octahedra, and the carriers of the superconducting current have been electron vacancies, or 'holes'. By contrast, we report here the discovery of a family of superconducting copper oxides in which the carriers are electrons. The new superconductors are Ce^{4+}-doped compounds, with the formula $Ln_{2-x}Ce_xCuO_{4-y}$, where Ln stands for the lanthanides Pr, Nd or Sm. The compounds have the Nd_2CuO_4 (T′-phase) structure[2], which is composed of sheets of Cu–O squares (see Fig. 1*a*). This structure has no apical oxygen atoms, in contrast to the T-phase structure with Cu–O octahedra (Fig. 1*b*), as observed in $La_{2-x}Sr_xCuO_4$ (refs 1, 2), and the T*-phase structure with Cu–O pyramids (Fig. 1*c*), as in $Nd_{2-x-z}Ce_xSr_zCuO_4$ (ref. 3 and Y. T. *et al.*, manuscript in preparation).

The superconducting materials were synthesized from a mixture of rare-earth metal oxides (CeO_2, Pr_6O_{11}, Nd_2O_3, Sm_2O_3) and CuO. The mixed powder was calcined in air at 950 °C for 10 hours, pressed into pellets and sintered in air at 1,150 °C for 12 hours. The samples were quenched in air to room temperature, and were found to be single-phase. To produce superconductivity, the Ce-doped samples were annealed for 10 hours under reducing conditions—at 1,000 °C in a stream of an Ar/O_2 gas mixture with $p_{O_2} < 10^{-3}$ atm—and quenched to room temperature in the same atmosphere. Best results were obtained with $p_{O_2} \approx 8 \times 10^{-5}$ atm.

Figure 1 shows the temperature dependence of resistivity for $Nd_{2-x}Ce_xCuO_{4-y}$. Although the undoped compound Nd_2CuO_4 is a typical semiconductor, doping with Ce^{4+} ions increases the conductivity (Y. T. *et al.*, manuscript in preparation). A remarkable decrease in resistivity was also observed in the undoped sample on quenching from 1,150 °C in air down to room temperature, which produces an oxygen deficiency ($y \approx 0.04$). These observations indicate that Ce^{4+}-doping and/or oxygen deficiencies introduce mobile electrons into the parent compound. The T′-phase Nd-based copper oxide cannot be doped with holes (Y. T. *et al.*, manuscript in preparation), whereas the T*-phase compound $Nd_{2-x-z}Ce_xSr_zCuO_4$ (Fig. 1*c*) can be doped with holes but not with electrons, which is also the case for the T-phase structure (Fig. 1*b*).

A fairly low resistivity ($\lesssim 10^{-2}\ \Omega$ cm) was observed for $Nd_{2-x}Ce_xCuO_{4-y}$ for $x = 0.15$, the sample showing semimetallic but not superconducting behaviour. (In Fig. 2, however, this sample shows a resistivity drop at around 9 K, which indi-

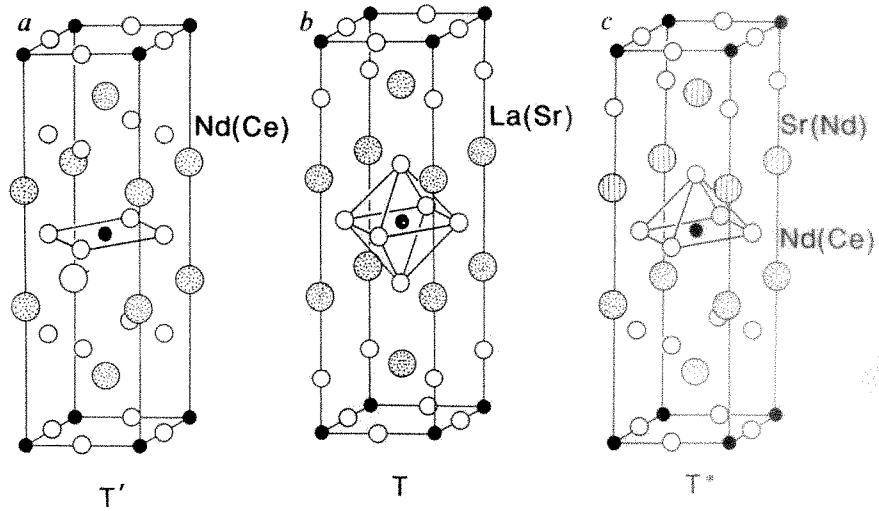


Fig. 1 Crystal structures of *a*, $Nd_{2-x}Ce_xCuO_4$ (T′-phase), *b*, $La_{2-x}Sr_xCuO_4$ (T-phase) and *c*, $Nd_{2-x-z}Ce_xSr_zCuO_4$ (T*-phase).

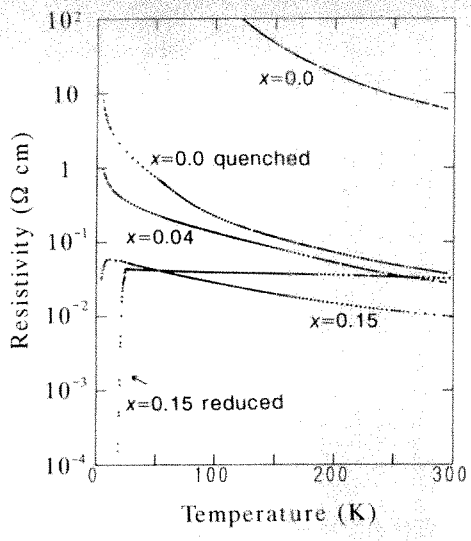


Fig. 2 Resistivity of $Nd_{2-x}Ce_xCuO_{4-y}$ as a function of temperature. In the $x = 0.15$ sample, after reduction, there is an onset of superconductivity at $T_c \approx 24$ K.

cates superconductivity in a small portion of the sample.) Bulk superconductivity in the $x = 0.15$ sample was achieved by the annealing procedure described above. Both resistivity and magnetization measurements indicated an onset of superconductivity at ~24 K. The concentration (q) of electrons introduced per [Cu-O] unit, or the effective copper valence ($2 - q$), was estimated by an iodometric titration technique[4] in which Ce^{4+} is reduced to Ce^{3+}. The $x = 0.15$ sample used to obtain Fig. 2 has $q = 0.20$ before the reducing procedure, and $q = 0.28$ after this procedure (that is, when it is superconducting). This corresponds to an increase in oxygen vacancies ($y \approx 0.07$). Therefore, an increase in electron density beyond a certain value ($q \approx 0.20$) seems to initiate superconductivity in which electrons are the charge carriers.

That the carriers are electrons was also confirmed by measurements of the Hall coefficient. The sample $Nd_{1.85}Ce_{0.15}CuO_{3.93}$, with $T_c = 24$ K, shows a negative Hall coefficient: $R_H = -6.5 \times 10^{-4}$ cm^3 C^{-1} at 300 K and -2.3×10^{-3} cm^3 C^{-1} at 80 K. This observation contrasts sharply with the other copper oxide superconductors so far known, in which positive Hall coefficients are always observed, indicating hole-type carriers.

The X-ray diffraction pattern for $Nd_{1.85}Ce_{0.15}CuO_{3.93}$ is shown in Fig. 3*a*, and, for comparison, those of the hole-superconducting T-phase $La_{1.85}Sr_{0.15}CuO_4$ (Fig. 3*b*) and T*-phase $Nd_{1.4}Ce_{0.2}Sr_{0.4}CuO_4$ (Fig. 3*c*) (see also Fig. 1). The diffraction pattern of $Nd_{1.85}Ce_{0.15}CuO_{3.93}$ is essentially identical to that of the undoped T′-phase compound Nd_2CuO_4, and all diffraction peaks discernible in Fig. 3*a* can be indexed assuming the lattice constants of the typical tetragonal T′-phase structure[2], $a = 3.95$ Å and $c = 12.07$ Å. The CuO_4 square in the T′-phase is considerably expanded and the c axis shrunk, relative to the T- and T*-phase structures, for which $a = 3.78$ Å, $c = 13.2$ Å, and $a = 3.85$ Å, $c = 12.5$ Å, respectively.

Electron-superconductivity is not restricted to the Nd-based compounds, but is also evident in the T′-phase of Pr- and Sm-based copper oxide compounds Ce-doped and reduced by the same procedure; again the electron concentration must be increased beyond $q = 0.20$, and superconductivity occurs at around 20 K. In Fig. 4 we show measurements of the Meissner effect in three different samples, $Ln_{1.85}Ce_{0.15}CuO_{4-y}$ (with Ln representing Pr, Nd and Sm), carried out using a SQUID magnetometer in an applied field of 10 Oe under field-cooled conditions. A substantial Meissner signal was observed in all of the compounds. In particular, the large signal for $Nd_{1.85}Ce_{0.15}CuO_{3.93}$—more than 25% of the ideal value—

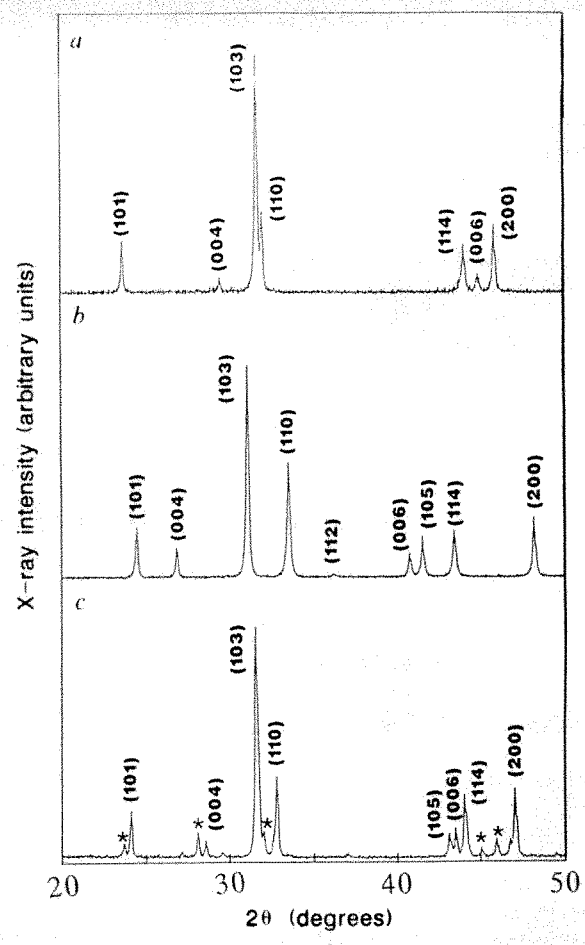


Fig. 3 Powder X-ray diffraction patterns of *a*, $Nd_{1.85}Ce_{0.15}CuO_{4-y}$, (T′-phase), *b*, $La_{1.85}Sr_{0.15}CuO_4$ (T-phase) and *c*, $Nd_{1.4}Ce_{0.2}Sr_{0.4}CuO_{4-y}$ (T*-phase). Asterisks indicate peaks from a secondary phase.

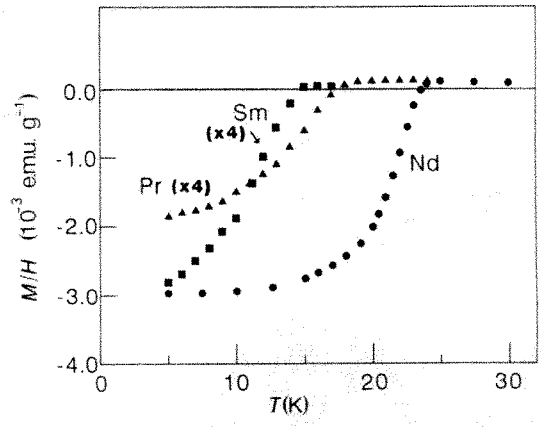


Fig. 4 Meissner signals in T′-phase $Ln_{2-x}Ce_xCuO_{4-y}$ (where Ln represents Pr, Nd or Sm). We plot the ratio of magnetization M to applied field H, for $H = 10$ Oe.

confirms the bulk nature of superconductivity. The observed T_c (24 K) is comparable with those in the corresponding hole-superconducting single-layer copper oxides: $T_c = 28$ K in the T*-phase (with Cu-O pyramids) and $T_c = 40$ K in the T-phase (with Cu-O octahedra).

The compound $Pr_{1.85}Ce_{0.15}CuO_{4-y}$ (Fig. 4) is the first example of a Pr-based copper oxide superconductor. Hole-doped Pr-based copper oxides, such as the T*-phase compound $Pr_{2-x-y}Ce_xSr_zCuO_{4-y}$ (Y. T. *et al.*, manuscript in preparation) and the 1-2-3 compound $PrBa_2Cu_3O_7$ (ref. 5), show neither metallic nor superconducting behaviour. This has been

attributed to the partial trapping of doped holes by Pr ions, or equivalently to the mixed-valent nature of Pr^{n+} ions ($3 < n < 4$). The appearance of superconductivity in $Pr_{2-x}Ce_xCuO_{4-y}$ further implies that the carriers are electrons in this T'-phase compound, because Pr^{3+} cannot be further reduced.

In conclusion, we have demonstrated for the first time that electron-doping in copper oxide compounds having the T'-phase structure, comprising CuO_2 sheets with no apical oxygen, gives rise to superconductivity. This observation may contain important implications for theories of high-T_c superconductivity.

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Unusually simple crystal structure of an Nd–Ce–Sr–Cu–O superconductor

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Recently, a new copper oxide superconductor with an onset temperature of 28 K was found in the Nd–Ce–Sr–Cu–O system[1]. The superconducting phase was identified by X-ray diffraction and electron microscopy[2], and the metal-atom positions are identical with those in the K_2NiF_4 or Nd_2CuO_4 structure[3], except for the order of the cations Nd(Ce) and Sr. Here we report the refinement of the crystal structure using neutron powder diffraction. We find that it comprises alternating slabs of the K_2NiF_4 and Nd_2CuO_4 structures, and that all the copper atoms are crystallographically identical, with oxygen in fivefold (square-pyramidal) coordination. In having only one kind of copper site, this new compound is structurally simpler than the other CuO_5-bearing superconductors such as $YBa_2Cu_3O_7$, and may therefore be particularly helpful in the effort to understand the origins of high-temperature superconductivity.

A sample with the composition $(Nd_{0.66}Sr_{0.205}Ce_{0.135})_2CuO_{4-\delta}$ was prepared by mixing Nd_2O_3, $SrCO_3$, CeO_2 and CuO in the appropriate ratio. The mixture was pressed into a pellet, reacted in air at 1,150 °C for 15 h, and then cooled to 900 °C. The reacted mixture was pulverized, pressed into a pellet and reheated under the same conditions. Finally, it was annealed at 550 °C for 12 h under a high pressure of O_2 (80 kg cm^{-2}), which, as has been observed before (ref. 2 and Y. Tokura *et al.*, preprint), increases both the superconducting volume fraction and T_c.

Our sample showed an onset of superconductivity, from magnetization measurements, at 22 K. The Meissner signal reached ~12% of the ideal value ($-1/4\pi$) at 8 K.

Initial structural parameters for use in a Rietveld analysis of the neutron data[4] were obtained by preliminary refinement of X-ray powder diffraction data. The X-ray data were measured at room temperature using a Rigaku diffractometer with rotating-anode source and graphite monochromator. Intensity data were collected with Cu Kα radiation at 0.02° intervals. The diffraction pattern indicated that the sample was nearly free from impurities. Rather low R factors were obtained: $R_{wp} = 12.9\%$, $R_p = 9.8\%$, $R_I = 8.6\%$, $R_F = 5.9\%$ and $R_e = 7.8\%$. The X-ray data were sufficient to determine the arrangement of the metal ions; a structure refinement on neutron powder diffraction data was carried out to obtain reliable structure parameters for oxygen.

Table 1 Structure parameters of $(Nd_{0.66}Sr_{0.205}Ce_{0.135})_2CuO_{4-\delta}$

Atom	Site	x	y	z	g	$10^3\beta_{11}$	$10^3\beta_{22}$	$10^3\beta_{33}$	B (Å^2)
M	2c	0	$\frac{1}{2}$	0.3895 (3)	1	—	—	—	0.47 (7)
M′	2c	0	$\frac{1}{2}$	0.1038 (3)	1	—	—	—	0.64 (7)
Cu	2c	$\frac{1}{2}$	0	0.2510 (3)	1	7 (1)	7 (1)	10 (2)	0.48
O(1)	4f	0	0	0.2374 (3)	1	11 (2)	17 (2)	24 (3)	1.05
O(2)	2c	$\frac{1}{2}$	0	0.4288 (5)	0.93 (2)	83 (5)	83 (5)	12 (4)	3.55
O(3)	2a	0	0	0	1	6 (2)	6 (2)	24 (4)	0.76

Estimated standard deviations in parentheses refer to the last digit. The temperature factor is defined as $\exp(-h^2\beta_{11} - k^2\beta_{22} - l^2\beta_{33})$. The occupation factors, g, of Nd, Ce and Sr are fixed at 0.49, 0.1 and 0.41 for site M, and at 0.83, 0.17 and 0.0 for site M′. Isotropic thermal parameters, B, were refined for sites M and M′; equivalent isotropic thermal parameters, B_{eq}, are given for sites Cu, O(1), O(2) and O(3).

Table 2 Interatomic distances, r, in $(Nd_{0.66}Sr_{0.205}Ce_{0.135})_2CuO_{4-\delta}$

Bonds	r (Å)	N*
M–O(1)	2.706 (2)	4
M–O(2)	2.7707 (6)	4
M–O(2†)	2.268 (4)	1
M′–O(1)	2.550 (2)	4
M′–O(3)	2.3231 (9)	4
Cu–O(1)	1.9355 (2)	4
Cu–O(2)	2.221 (3)	1

* Number of equivalent bonds.
† Coordinate triplet: y, $1-x$, $1-z$.

The neutron diffraction data were obtained on a high resolution time-of-flight neutron powder diffractometer, HRP, at the KENS pulsed spallation neutron source at the National Laboratory for High Energy Physics. The specimen was contained in a cylindrical vanadium cell. The scattering angle, 2θ, was fixed at 170°, and intensity data were collected at room temperature for 20 h, using a gate width of 4 μs. Intensity data corresponding to interplanar spacings, d, between 0.51 and 3.37 Å (7,012 points) were used for Rietveld refinements. Preferred orientation was corrected on the assumption that the cleavage plane is (001), using a gaussian preferred-orientation function.

The observed data are consistent with just one of the three models proposed by Takayama-Muromachi *et al.*[2]. Final structure parameters are listed in Table 1, and selected metal–oxygen distances in Table 2. The refined lattice parameters are $a = 3.8563\ (3)$ Å and $c = 12.4842\ (9)$ Å. R factors were $R_{wp} = 5.9\%$, $R_p = 4.5\%$, $R_I = 3.5\%$, $R_F = 2.7\%$ and $R_e = 4.8\%$. Figure 1 shows the final profile fit and difference patterns. The good agreement between observed and calculated patterns strongly supports the proposed structural model.

Because the occupation factor, g, and thermal parameters for site O(2) are considerably correlated, these parameters cannot be determined very accurately. However, taking $g = 1$ for site O(2) gave larger R factors and a larger thermal parameter ($B_{eq} = 4.26$), suggesting that this site is slightly oxygen-deficient. The oxygen stoichiometry $4-\delta$, calculated from the measured g, is 3.93. The increase in superconducting volume fraction and in T_c that accompanies oxygen annealing presumably results from an increase in occupation of this site.

The equivalent thermal parameter of site O(2) was fairly large: $B_{eq} = 3.55$ Å^2. The anisotropic thermal parameters U_{11} and U_{33}

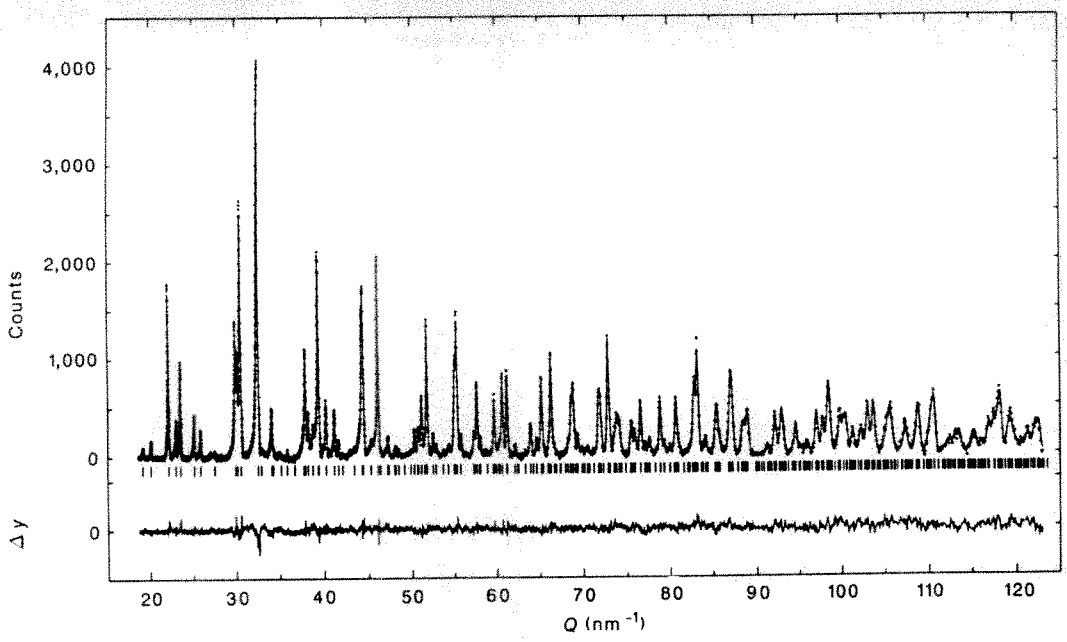


Fig. 1 Rietveld refinement of a time-of-flight neutron diffraction pattern for $(Nd_{0.66}Sr_{0.205}Ce_{0.135})_2CuO_{4-\delta}$. Scattering vector $Q = 2\pi/d$, where d is the interlayer spacing. The solid lines are calculated intensities, crosses indicate experimental data, and Δy is the difference between the two. The short vertical lines mark the positions of 501 peaks.

are, respectively, 0.063 Å^2 and 0.0092 Å^2, indicating that the thermal ellipsoid of O(2) expands perpendicular to the c axis. This marked anisotropy of thermal vibration is understandable in view of the relatively short Cu-O(2) and M-O(2) bonds parallel to the [001] direction. In addition, the large value of U_{11} for this site must be partly due to the random distribution of Nd, Ce and Sr atoms at site M.

The coordination numbers of M, M′ and Cu are, respectively, 9, 8 and 5. Sr^{2+} ions, which are larger than Nd^{3+} or Ce^{3+} (or Ce^{4+}) ions, selectively occupy the 9-coordinate site at $(0, \frac{1}{2}, \sim 0.39)$. The average M-O distance (2.69 Å) is much larger than that of M′-O (2.44 Å). The CuO_5 pyramid contains four short Cu-O(1) bonds (1.9355 Å) and one long Cu-O(2) bond (2.221 Å), but the Cu-O(2) bond is shorter than those in other superconductors with such CuO_5 pyramids, such as $YBa_2Cu_3O_7$ and $Tl_2CaBa_2Cu_2O_8$. The valence of Cu, calculated using the Brown-Altermatt formula[5], is 2.23. The bases of the pyramids form a two-dimensional CuO_2 plane perpendicular to the [001] direction. The M-O(2) bond parallel to the [001] direction is only 2.268 Å, which is comparable with the Cu-O(2) distance.

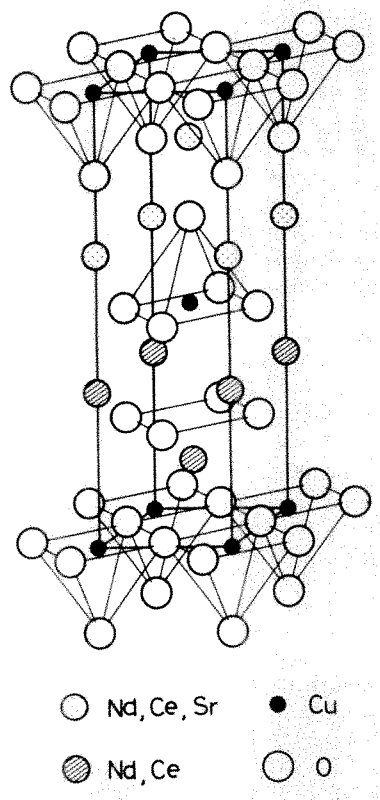


Fig. 2 Crystal structure of $(Nd_{1-x}Sr_{x-y}Ce_y)_2CuO_{4-\delta}$.

The crystal structure of $(Nd_{1-x}Sr_{x-y}Ce_y)_2CuO_{4-\delta}$ is shown in Fig. 2. The unit cell consists of two parts: the upper part in Fig. 2 corresponds to the K_2NiF_4 structure, in which the M (Nd, Ce, Sr) ion is coordinated to nine oxygen atoms, and the lower part exhibits the Nd_2CuO_4 structure, incorporating M′(Nd, Ce) ions in eightfold coordination. All of the copper atoms are in a single crystallographic site, with square-pyramidal coordination. High-T_c superconductors are classified into two structural groups, those that contain copper in distorted octahedral coordination (for example, $(La_{1-x}M_x)_2CuO_4$ (where M is Ca, Sr or Ba) and $(Bi, Tl)_2(Sr, Ba)_2CuO_6$) and those that contain copper in pyramidal coordination (for example, $LnBa_2Cu_3O_7$ (where Ln is Y or various lanthanide elements), $(Bi, Tl)_mCa_n(Sr, Ba)_2Cu_{n+1}O_{m+2(n+2)}$ and $Pb_2Sr_2ACu_3O_{8+\delta}$ (where A represents Ln or Ln+(Sr or Ca))[6]). Clearly, $(Nd_{1-x}Sr_{x-y}Ce_y)_2CuO_{4-\delta}$ has the simplest structure of the 'pyramidal' superconductors.

Here we have assumed that Ce^{3+} ions occupy both the M and M′ sites. We are now examining the local and electronic structures of Ce by extended X-ray absorption fine structure (EXAFS) and X-ray photoelectron spectroscopy (XPS), with the aim of determining the distribution of Ce between these two sites. We are also investigating the apparent sensitivity of superconductivity to the Nd/Sr/Ce ratio.

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Experimental and theoretical equation of state of cubic boron nitride

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The phase diagram of boron nitride (BN) is similar to that of carbon, incorporating phases at high temperatures and pressures whose structures and physical properties resemble diamond[1–9]. Cubic zinc-blende-structured BN is especially important because it is extremely hard—second only to diamond. Here we report the first measurement of the 300-K equation of state of this material to ultrahigh pressures (115 GPa), and obtain a zero-pressure bulk modulus of 369 ± 14 GPa. A theoretical equation of state derived from first-principles pseudopotential calculations yields a 300-K isotherm that agrees with our experimental results to better than 2.5% in volume and 2.0% in bulk modulus. The high-pressure Hugoniot (shock-wave equation of state) calculated from our equation of state for BN is in good agreement with existing shock-wave data. Our study illustrates the reliability of current experimental techniques (such as the ruby-fluorescence calibration) and theoretical methods (pseudopotentials) for characterizing the behaviour of superhard, incompressible materials under high pressure.

At ambient conditions, BN exists in a hexagonal structure which is analogous to that of graphite. At pressures above 4.5 GPa and temperatures above 1,500 K, BN exhibits two polymorphs, corresponding to the diamond (cubic zinc-blende-structured) and lonsdalite (hexagonal wurtzite-structured) forms of carbon. Both the structure and the physical properties of zinc-blende-structured BN, the thermodynamically stable phase at high pressure, resemble those of diamond. Because of its extreme hardness, its chemical inertness, its high melting temperature (>3,000 K) and its high thermal conductivity (~200 $W m^{-1} K^{-1}$), cubic BN is commercially important[10]. But despite these unusual physical properties, there has been no direct experimental measurement of its equation of state. This lack of data is surprising because the value of the bulk modulus, for example, is of considerable importance in understanding the relationship between a crystal's structure, hardness and stability—information that can help in the design of new superhard materials with technological potential. In addition, the equation of state offers a powerful way of comparing the reliability and predictive power of current theoretical and experimental techniques for characterizing the physical properties of condensed matter. We have determined the equation of state of cubic BN to pressures in excess of 100 GPa by both experimental and theoretical means.

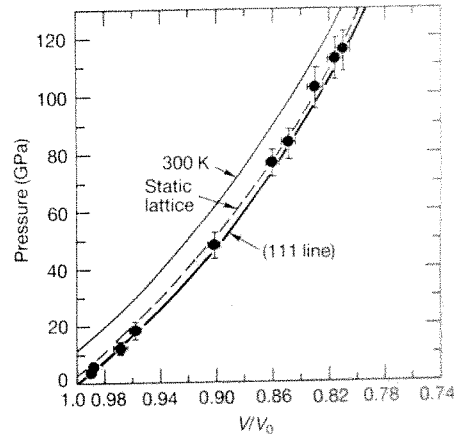


Fig. 1 Experimental pressure–volume data for cubic BN at 300 K (circles with error bars) are compared with theoretical isotherms for the static lattice (dashed curve) and for BN at 300 K (thin solid line). The thick solid line is the Birch–Murnaghan (third-order Eulerian finite strain) isotherm that is obtained from a least-squares fit to our static compression measurements. The reference volume used here is the volume measured at ambient conditions, V_0 = 7.113 $cm^3 mol^{-1}$, so the theoretical and experimental results are compared here in terms of absolute volume.

Table 1 High-pressure X-ray diffraction data for cubic BN

Run no.*	Pressure (GPa)	Lattice parameter† (Å)	Volume V/V_0
090287a	3.2 (±0.2)	3.605 (±0.002)	0.992 (±0.001)
090987a	6.5 (±0.4)	3.594 (±0.003)	0.983 (±0.002)
091587a	12.5 (±2.1)	3.574 (±0.006)	0.967 (±0.004)
092587a	17.9 (±3.0)	3.564 (±0.005)	0.958 (±0.004)
101187b	48.0 (±4.2)	3.492 (±0.006)	0.901 (±0.005)
102187b	76.5 (±4.7)	3.438 (±0.006)	0.860 (±0.005)
111787b	82.8 (±5.2)	3.422 (±0.005)	0.848 (±0.004)
120887b	101.8 (±6.8)	3.399 (±0.006)	0.831 (±0.005)
123087b	112.0 (±7.3)	3.378 (±0.006)	0.816 (±0.005)
010788b	115.6 (±7.1)	3.370 (±0.005)	0.810 (±0.004)

* a and b designate different samples.
† The lattice parameter is from the (111) diffraction line only. All other diffraction lines of cubic BN have intensities of less than 10% of the intensity of this line and therefore are not strong enough to be observed at high pressure.

The sample of cubic BN used in this study was supplied by R. C. DeVries of General Electric Corporation and comprised single crystals 30–50 μm in size. The lattice parameter, determined by X-ray diffraction at zero pressure, is 3.615 ± 0.002 Å. For the high-pressure experiments, gasketted samples of cubic BN were compressed in a Mao–Bell-type diamond cell between diamonds with 200-μm culets[11]. The sample region was typically 100 μm in diameter and 10 μm thick at high pressures. A 4:1 mixture of methanol:ethanol was used as a pseudohydrostatic pressure medium, and between two and five small pieces of powdered ruby were included with the BN sample for pressure measurement using the ruby-fluorescence technique[12,13]. A Mo K_α X-ray beam was collimated to a diameter of 80 μm and directed through the high-pressure samples along the axis of force of the diamond cell. X-ray diffraction patterns were recorded at pressures of between 0 and 115 GPa with typical run times of 100–150 hours.

The experimentally measured lattice parameters and volumes of cubic BN under pressure are listed in Table 1, and the isothermal equation of state is plotted in Fig. 1. To determine the zero-pressure values of the isothermal bulk modulus (K_0) and its pressure derivative (K_0'), we have analysed the data using a Birch–Murnaghan (third-order Eulerian finite-strain) equation of state[14]. Despite the large value of K_0 (369 ± 14 GPa), we have determined it and K_0' with considerable precision because of the high pressures that were reached[15].

The static structural properties of cubic BN were calculated using a first-principles total-energy approach[16] within a localized-orbital formalism[17]. The localized basis provides a more convenient representation than a plane-wave basis, which had been used in a previous calculation[18], because the valence states of BN are highly localized by the large depth of the angular-dependent components of the boron and nitrogen pseudopotentials. The formalism that we used is described in detail elsewhere[16,17], and as far as possible we chose the same approximations here as were used before[18–22]. To facilitate the evaluation

of matrix elements, the angular-dependent components up to $l=2$ were expanded in terms of gaussian functions using a Monte Carlo simulated-annealing method[23]. Up to 12 gaussians were used in the expansion of the sharp p-potentials, which resulted in fitting errors smaller than 0.01 Rydbergs. The wave-functions are expanded in a basis of Bloch sums of gaussian orbitals $f(r)$ centred on the atomic sites, which have the form:

$$f(r)=e^{-\alpha r^2}r^l Y_{l,m}(\Theta,\Phi)$$

Four and five even-tempered values of α are used for B and N respectively, and values of $l=0$ and 1 are included, so that there are 36 basis functions per molecule. The gaussian decays α for B and N were chosen by fully minimizing the total energy with respect to the smallest and largest αs in the even-tempered series. They are 0.23 and 5.80 for N, and 0.17 and 5.3 for B. Inclusion of states with d-symmetries shifted the energies for the equilibrium volume almost uniformly by ~0.1 eV per atom pair. This small change is typical of first-row elements, whose energy separation between the atomic d-states and the Fermi level is very large. The crystal potential is iterated to full self-consistency. To perform the summations in the Brillouin zone, the same ten k-point samplings as used in the earlier calculations were chosen[18].

The results of our calculations on cubic BN are shown in Fig. 2 and summarized in Table 2. The static-lattice energy was obtained at each of 12 volumes between 50 and 90 a.u.^{3} (1 atomic unit (a.u.) = 0.529 Å). To compare the results with 300-K data, we used the Debye model to derive the zero-point energy and the internal-energy difference between 0 K and 300 K. Our approach in making these vibrational corrections to the lattice energy is purely empirical: we use the experimental values of $\theta_D=1{,}800$ K for the Debye temperature and $\gamma_0=1.87$ for the Grüneisen parameter[10,24] referenced to the volume measured at ambient conditions, $V_0=79.78$ a.u.^{3}. Also, we assume that γ/V is constant (see, for example, ref. 25). Because of the large θ_D of BN the difference between lattice energies and static-lattice energies is almost entirely due to the zero-point energy, and the effect of compression is to increase the vibrational correction (through the effect on γ) so that the equilibrium volumes at 0 K and 300 K are larger than the volume for the static lattice.

The equilibrium properties derived from the calculations depend in detail on the form of the equation of state that is used to fit the energy–volume points. In Fig. 2 we have assumed that the energy is a third-order polynomial in the Eulerian finite-strain, as we did in our analysis of the experimental measurements[14], because this is identical to fitting pressure–volume data with the Birch–Murnaghan equation of state. Thus, we are treating our theoretical and experimental results identically, iteratively solving to obtain V_0 for the best-fitting equation of energy as a function of strain. We obtain values of 3.625 Å and 3.648 Å for the lattice parameters of the static and 300-K lattice, respectively, which agree with experiment to better than 1% (Table 2).

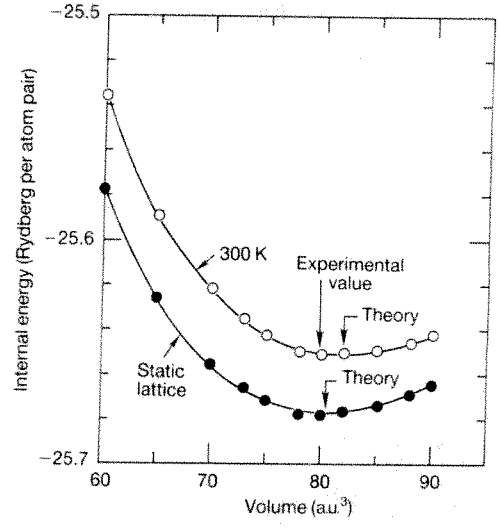


Fig. 2 Theoretical results for the internal energy (Rydberg per molecule) as a function of volume (in atomic units) of cubic BN. The static lattice energies (solid circles) are calculated using the LCAO pseudopotential approach. Corrections for zero-point energy and for the thermal energy between 0 K and 300 K were obtained from the Debye model, and the open circles represent the total energy at 300 K. The two curves show Eulerian finite-strain fits to the static-lattice and 300-K points, with the difference in energy between 0 K and 300 K being within the width of the 300 K curve. Arrows labelled Theory indicate the theoretical equilibrium volumes for the static lattice and at 300 K. These are derived from the fitted curves and are compared with the experimentally determined value for the volume at 300 K.

Our results compare favourably with the previous calculations using a plane-wave basis[18]. If a Murnaghan equation of state is used to fit the calculated points, as had been done in the earlier work, the differences between the equilibrium properties calculated for the static lattice for localized and plane-wave bases are small relative to the uncertainties (Table 2). These differences are comparable to the variation caused by changing the form of the equation of state used. In addition, absolute uncertainties are dependent on the convergence achieved and on the formulation used for the exchange and correlation energy (for example, the Hedin–Lundqvist expression[26]). Nevertheless, the high level of convergence achieved here is reflected in the cohesive energy (Table 2), which is slightly greater than was

Table 2 Comparison of the properties of cubic BN from experiment and theory

	Lattice parameter (Å)	K_0 (GPa)	K_0'	Cohesive energy E_{co} (eV per atom pair)	
Experiment (300 K)					
Static comp. (0–115 GPa)	3.615 (±0.002)	369 (±14)	4.0 (±0.2)	—	Birch fit[14]
Theory (Static lattice: 0 K)					
LCAO*	3.625 (±0.001)	368	3.6	—	Birch fit[14]
LCAO*	3.618 (±0.008)	370 (±10)	3.1 (±0.1)	13.5 (±0.1)†	Murnaghan fit[34]
PW‡	3.611	367	3.05	13.3	Murnaghan fit[34]
Theory (300 K)					
LCAO*	3.648 (±0.001)	363	3.5	—	Birch fit[14]

* Linear-combination-of-atomic-orbitals basis: this work.

† The experimental value for the cohesive energy of hexagonal BN is 13.0 eV (ref. 32). The cohesive energy for the zinc-blende phase should differ from this by ~0.1 eV (ref. 33). The somewhat larger value obtained theoretically is typical of calculations using the local-density approximation.

‡ Plane-wave basis as in ref. 18.

obtained in the plane-wave calculation. Apparently, using a finite number of plane waves in the latter calculations leads to a reduced estimate of the cohesive energy.

In Table 2 and Fig. 1, we compare the experimental and theoretical equations of state for cubic BN. The theoretical isotherms are obtained by adding the zero-point and thermal pressure derived from the Debye model to the negative volume-derivative of the static-lattice energy curve shown in Fig. 2. It is clear that although the calculated volume is slightly (but systematically) large, by 2.0–2.5% relative to the measured values, the theoretical equation of state is in excellent agreement with our experimental isotherm. In fact, the derived parameters K_0 and K_0' are identical, given the uncertainties and approximations involved (Table 2). We conclude, therefore, that the high-pressure compression of cubic boron nitride is well characterized by these theoretical and experimental results, confirming the predictions of our earlier study[18].

To check the reliability of our equation of state for cubic boron nitride, we compare our results with independent shock-wave (Hugoniot) measurements[5,27]. The advantage to the latter is that whereas static experiments depend on the ruby-fluorescence technique for calibrating pressures, the Hugoniot data represent direct measurements of volumes and pressures, free from any pressure calibration. However, the primary difficulties involved in interpreting shock-wave data are: (1) the need to apply thermal corrections and to account for initial sample porosity; (2) the possible influence of strength effects; and (3) possible kinetic hindrances in the case of shock-induced phase transitions.

The existing Hugoniot data for BN are obtained from polycrystalline samples that are initially in the low-pressure (hexagonal) polymorph, with variable porosity ranging between 5 and 10%. We calculate Hugoniots assuming that the hexagonal BN transforms entirely to the cubic phase (that is, that there is no kinetic hindrance) and that strength effects can be neglected. If either assumption is wrong, the actual Hugoniot states would be at pressures (or shock-wave velocities, U_s) greater than our calculated values for a given volume or particle velocity (u_p). The calculations are carried out using the Mie-Grüneisen and Eulerian finite-strain formalisms, with both initial porosity and the energy of transformation being taken into account[25,28].

Using the parameters listed under Fig. 3, we predict the high-pressure Hugoniot for BN in terms of U_s as a function of u_p. Although we cannot prove that cubic BN, rather than another phase or a mixture of phases, is formed at high shock pressures, the good agreement between the calculated and observed Hugoniots does support this inference. There is no evidence for strength effects or for partial transformation at $u_p > 2\ \mathrm{km\ s^{-1}}$, except in the pyrolitic samples of ref. 5. Even the curvature of the U_s–u_p trend is reproduced by our calculations (Fig 3). According to our model this curvature is due mainly to the initial sample porosity, although the experimental curvature may exceed that predicted above $u_p = 4.2\ \mathrm{km\ s^{-1}}$. We concur with Gust and Young[5] that the samples may be molten at these high shock pressures. For example, we estimate Hugoniot temperatures of 5,000–5,500 K at 100 GPa for the porous samples shown in Fig. 3; these values of temperature are probably low because they are obtained assuming that the specific heat is equal to the Dulong–Petit value[5]. Hence, shock-induced melting is a plausible explanation for the observed U_s being somewhat lower than our predicted values at Hugoniot pressures above 85 GPa ($u_p > 4.2\ \mathrm{km\ s^{-1}}$). As a result, we conclude that the shock-wave data are in good agreement with the equation of state that we have derived by static-compression measurements and by linear-combination-of-atomic-orbitals pseudopotential calculations. This provides implicit additional support for the validity of the ruby-fluorescence calibration used in static-compression experiments.

Previous empirical estimates of the bulk modulus of cubic BN range up to 465 ± 50 GPa. This value, based on the interpolation of empirical correlations for elastic constants, is clearly too high[29]. In contrast, a scaling argument in which the bulk modulus for diamond- and zinc-blende-structured compounds is inversely proportional to a power of the bond length leads to an estimate of 367 GPa for cubic BN (ref. 30). This is in close agreement with both our experimental and theoretical values of K_0, and it gives a prediction of K_0' that is close to our observed value of 4 (ref. 31). Furthermore, the scaling argument successfully reproduces the bulk moduli of a number of diamond- and zinc-blende-structured solids, including carbon (435 GPa), silicon (98 GPa) and germanium (76 GPa). The success of this scaling is consistent with the idea that shorter bond distances and smaller heteropolarities are associated with larger bulk moduli, and hence larger bond strengths and greater hardness, in tetrahedrally bonded solids.

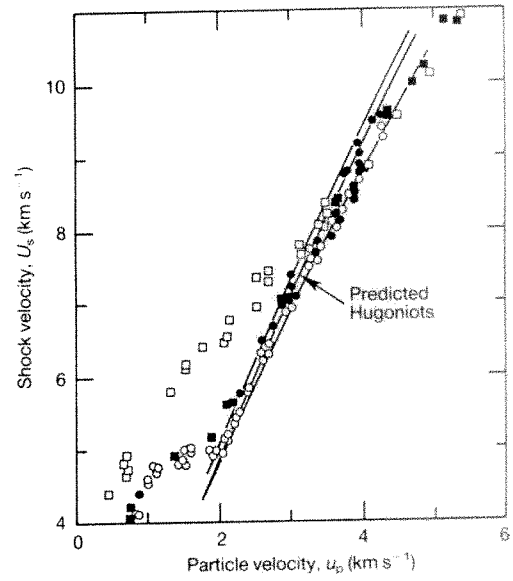


Fig. 3 Comparison of shock-wave data on BN with Hugoniots predicted from the present equation of state for cubic boron nitride. Open and closed squares represent the pyrolitic and hot-pressed samples of ref. 5, whereas the open and closed circles represent samples of ref. 28 with initial densities of 2.08 Mg m^{-3} and 2.11–2.15 Mg m^{-3}, respectively. The middle curve corresponds to the Hugoniot derived from the following parameters (notation as in ref. 28): $\rho_{00} = 2.10$ (±0.05) Mg m^{-3} (refs 5, 35), $\rho_{01} = 2.258$ (±0.001) Mg m^{-3} (ref. 35), $\rho_{02} = 3.489$ (±0.001) Mg m^{-3} (ref. 35), $K_{s02} = 371$ (±14) GPa, $dK_{s02}/dP = 4.0$ (±0.2), $\gamma_{02} = 1.87$ (±0.322) (refs 10, 24), $d\ln\gamma/d\ln V = 1$ and $E_{tr} = 5$ (±3) kJ mol^{-1} (ref. 10). The outer curves reflect the estimated uncertainties in the Hugoniot parameters.

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Evidence from Lewisian limestones for isotopically heavy carbon in two-thousand-million-year-old sea water

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A number of 2,000-million-year-old marbles have extraordinarily heavy carbon isotope signatures ($\delta^{13}C \approx +12‰$ PDB)[1,2], suggesting that there was a worldwide excursion in the carbon isotope composition of sea water at this time. To test this hypothesis we have measured the carbon isotope signatures of 2,000-million-year-old amphibolite facies marbles from the Scottish Lewisian. The occurrence of a major isotope excursion is confirmed by the heavy carbon isotope signatures of these rocks ($\delta^{13}C$ up to +13‰ PDB), which we attribute to an origin contemporaneous with sedimentation. This excursion coincides with a major change in the redox state of the oceans which is likely to have induced considerable changes in the carbon cycle. We suggest that the excursion was due to increased rates of organic carbon deposition resulting from an increase in organic productivity.

Supracrustal rocks from the Loch Maree Group of the Lewisian were deposited after the main 2.7-Gyr granulite facies metamorphic event. These rocks include marbles, graphite schists, iron formations and quartzofeldspathic gneisses which underwent amphibolite facies metamorphism at 1.8 Gyr. The depositional age is unlikely to exceed 2.1 Gyr on the basis of the low initial $^{87}Sr/^{86}Sr$ ratio at the age of metamorphism[3], and of Sm–Nd crustal-residence ages[4]. The marbles include coarsely crystalline and microbrecciated varieties. Both quartz-present assemblages and quartz-absent, dolomite-bearing (such as tremolite–dolomite–calcite[5]) assemblages occur. Carbon and oxygen isotope compositions of calcites extracted from these marbles are shown in Fig. 1 and Table 1. A range of $\delta^{13}C$ and $\delta^{18}O$ values is found which may be attributed to interaction of marbles, initially containing heavy carbon and oxygen, with varying amounts of metamorphic fluid. Many samples containing heavy carbon also preserve oxygen isotope compositions similar to pre-metamorphic values. This is good evidence for a pre-metamorphic origin of the heavy carbon isotope signature, as any process involving a carbonate during metamorphism is likely to decrease its $\delta^{18}O$ value. Exchange with other silicates,

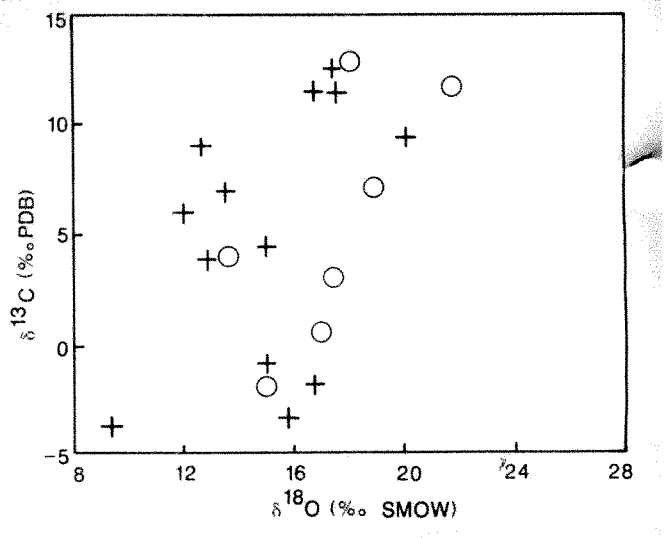


Fig. 1 $\delta^{13}C$ (relative to PDB) versus $\delta^{18}O$ (relative to standard mean ocean water (SMOW)) for analysed calcites. Circles represent Loch Maree data, and crosses are Gairloch data.

exchange with an infiltrating fluid and devolatization reactions are all likely to cause decreases in $\delta^{18}O$. Hence high-$\delta^{18}O$ calcites must have been little affected by metamorphic processes and should therefore preserve pre-metamorphic $\delta^{13}C$ (see, for example, ref. 6). In general, it is hard to conceive of metamorphic processes likely to increase $\delta^{13}C$ of a marble by more than a few parts per thousand. Metamorphism normally results in decreases in calcite $\delta^{18}O$ and $\delta^{13}C$ (ref. 6). We conclude that marbles from the Lewisian had $\delta^{13}C$ values of ~12‰ over a large area and that this signature is attributable to some synsedimentary process.

During the Phanerozoic, carbon isotope variations in marine limestones did not generally deviate by more than about 4‰ from the present $\delta^{13}C$ value of 0‰ (ref. 7). There is evidence, however, that during certain periods within the Precambrian many limestones had $\delta^{13}C$ values in excess of +5‰ (refs 8–12). A summary of carbon isotope variations in Precambrian marine limestones is shown in Fig. 2. Further data, not plotted in Fig. 2, exist for the period 1.0–0.5 Gyr which supports the occurrence of a series of positive excursions in seawater $\delta^{13}C$ at this time[12]. Of particular note are the extraordinarily ^{13}C-enriched marbles from the Lomagundi Province of Zimbabwe. Here dolomitic marbles associated with graphite-rich metasediments have isotope compositions of up to $\delta^{13}C = +12‰$. These marbles were deposited between 1.95 and 2.65 Gyr ago[1]. Recently, ^{13}C-enriched marbles with $\delta^{13}C$ of up to +12‰ have been found in Lofoten-Vesterålen, Norway. These were deposited between 1.8 and 2.1 Gyr ago in association with graphite schists and iron formations[13]. The marbles have experienced amphibolite facies metamorphism, but the heavy carbon isotope signatures have been attributed to pre-metamorphic processes on the basis of arguments identical to those above (ref. 2 and A.J.B. and A.E.F., manuscript in preparation). Heavy carbon isotope signatures have also been recorded in 2.0-Gyr-old marbles from Finland (ref. 8 and J. Karhu, personal communication) and in marbles of similar age from Soviet Karelia[14]. Interpretation is hampered by the poor resolution of the geochronological data (see Fig. 2), but it appears that ^{13}C-enriched limestones were particularly common about 2.0 Gyr ago. It is not clear whether this represents a single positive excursion in $\delta^{13}C$ or a series of excursions analagous to those described for the period 1.0–0.5 Gyr (ref. 12). The latter excursions are associated with changes in the $\delta^{34}S$ of marine sulphate. This may be explained in terms of maintenance of the redox cycle of the hydrosphere by the carbon and sulphur cycles[15]. A sulphur isotope excursion has not been

observed for the 2.0-Gyr $\delta^{13}C$ anomaly[11,16]. At this time, however, the oxygen cycle may have been controlled by coupling between the carbon and iron cycles rather than between the carbon and sulphur cycles[15]. Secular variations in the carbon isotope ratios of marine carbonates between 1.0 and 2.0 Gyr are as yet poorly constrained; this may be a period during which sea water had 'normal' carbon isotope signatures close to 0‰.

The 2.0-Gyr excursion could be attributed to a number of processes. It could represent a worldwide excursion in seawater carbon isotope composition, excursions in sea water restricted to localized basins or the operation of some process restricted to particular intervals within the geological record. Today, ^{13}C-enriched carbonates may be deposited from CO_2 derived from zones of anaerobic diagenesis[17] or from hot spring waters[18]. The former process is unlikely to explain the isotope systematics of carbonate-rich sediments and the latter is unlikely to explain the deposition of carbonate over large areas, restricted to particular intervals of geological time. Moderately ^{13}C-enriched carbonates are found in some evaporitic environments[19]. Evaporites are not, however, restricted to particular intervals within the Precambrian[20]. Sedimentary sulphates do occur as accessory minerals in some Lewisian and Lomagundi sediments, but extensive evaporite deposits are absent.

The carbon isotope compositions of marine limestones are generally controlled by the proportion of seawater carbon that passes into limestones and the proportion that passes into organic carbon (see, for example, refs 12, 21). Increases in the amount of organic carbon deposited can lead to positive $\delta^{13}C$ excursions, as during Phanerozoic anoxic events (ref. 22, for example). High-$\delta^{13}C$ limestones from Lomugundi, the Lewisian and Lofoten-Vesterålen are associated with organic-carbon-rich metasediments. We suggest therefore that the positive anomaly is associated with an increase in organic-carbon deposition in sediments either on a worldwide basis or within many marginal basins. Return to the deposition of carbonates with more normal isotope compositions could have been achieved by the operation of a number of buffering processes, perhaps including an increase in the rate of organic-carbon recycling within the oceans.

It appears that high-$\delta^{13}C$ carbonates deposited ~2 Gyr ago were deposited approximately synchronously with the transition from a reducing to an oxidizing hydrosphere[20]. The end of uraninite-conglomerate deposition and changes in the oxide minerals found in fluvial deposits and palaeosols suggest that a large increase in quantities of atmospheric oxygen occurred between 2.5 and 2.0 Gyr ago[20]. The maximum rate of deposition of iron formations occurred in this period, but declined sharply thereafter, reflecting the transition to a more oxidizing environment[20]. It has been suggested that similar increases in atmospheric oxygen accompanied the deposition of ^{13}C-enriched limestones between 1.0 and 0.5 Gyr (ref. 12). Increases in atmospheric and oceanic oxygen at about 2.0 Gyr are likely to have been accompanied by major changes in the carbon cycle. We suggest that increased oxygen contents of the oceans were produced and were accompanied by an increase in organic productivity. The increased oxygen content of the atmosphere may have been the direct result of an increase in the population of photosynthesizing bacteria[23]. Increasing oceanic oxygen contents may also have enabled the further exploitation of certain oceanic environments and enlargement of the biomass. This increased organic productivity, in the absence of compensating increases in the rate or organic-carbon recycling, would have increased rate of organic-carbon deposition and resulted in the deposition of high-$\delta^{13}C$ limestones.

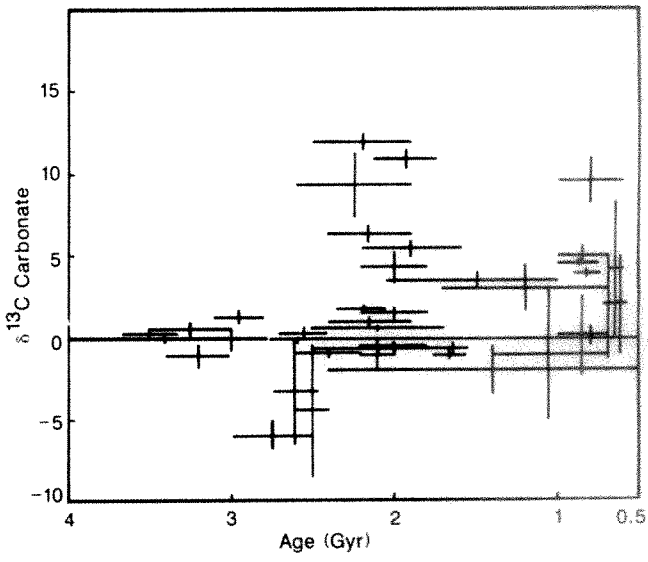


Fig. 2 $\delta^{13}C$ of Precambrian sedimentary carbonates plotted against age of deposition. Data from refs 1, 2, 8, 9, 10, 11 and this study.

Table 1 Calcite isotope data for Loch Maree Group supracrustals

Specimen number	National grid reference	$\delta^{13}C$ (‰ PDB)	$\delta^{18}O$ (‰ SMOW)
Loch Maree area			
S72588	NH012656	4.0	13.6
S72591	NH996671	−1.4	14.8
S72600	NG896768	0.5	16.9
S72601	NG903768	12.9	18.0
S72604	NG944760	11.7	22.0
S72605	NG944760	7.2	18.9
S72606	NG944760	3.1	17.5
Gairloch area			
S72609	NG866731	−3.9	9.4
S72613	NG856722	5.8	11.8
S72614	NG856722	3.9	12.8
S72615	NG818720	12.0	17.5
S72616	NG818720	12.1	17.7
S72617	NG838700	8.8	12.8
S72618	NG838700	12.8	17.5
S72620	NG836702	4.7	14.8
S72621	NG828712	7.2	13.6
S72622	NG811725	9.4	21.0
S72627	NG847716	−3.3	15.7
S72632	NG837730	−1.6	16.9

S numbers are British Geological Specimen numbers.

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Relationships between pre-rift structure and rift architecture in Lakes Tanganyika and Malawi, East Africa

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Continental rift systems are rips in plates caused by focusing of extensional stresses along some zone. In the same way that tensile cracks in the side of a brick building generally follow the mortar between bricks, rifts initially follow the weakest pathways in the pre-rift materials. There has even been a suggestion that the occurrence of rifts is controlled by pre-rift structure[1-3], although many workers do not subscribe to such direct causality. The relationships between pre-rift structure and rifting tend to become progressively more obscure as the scale of examination decreases, mainly because unravelling these relationships requires more precise information about the architecture of rifts, the nature of the pre-rift fabric and about the principal axes of stress than is generally available. Recent work by Project PROBE on the Tanganyika[4-15] and Malawi Rift Zones[12,16-22] permits crucial aspects of these inter-relationships to be examined in a more rigorous fashion than was previously possible. In this region, the rift system follows Proterozoic mobile belts and bifurcates around the Tanganyika craton, which apparently has acted as a resistant core (Fig. 1). Here we show that the fundamental architecture of the Tanganyika and Malawi Rift Zones (that is, the positioning of half-grabens, the way they link together, and the types and trends of linking structures) is strongly influenced by the nature of the pre-rift fabric and the orientation of the stress field.

Fault-pattern maps of the Lakes Tanganyika and Malawi Rift Zones are shown in Fig. 2, and generalized structure maps in Fig. 3. Both rift zones are composed of basins that are asymmetric in cross-section. The deep sides of the basins are bounded by border-fault systems (BFSs), along which subsidence tends to be cumulatively greater than elsewhere in the basin. In plan view the BFSs are mapped as slightly to markedly arcuate. Subsidence varies along BFSs, creating an asymmetry in the strike direction that complements the dip asymmetry. These asymmetric basins, or 'half-grabens'[8,11,23], link together in various ways.

The general theme of linking is that adjacent half-grabens are offset from each other in the strike dimension and face in opposite directions, often resulting in a roughly sinusoidal alternation of dip polarities along strike[4-8]. The best examples of this occur between the Livingstone-Usisya and Usisya-Metangula half-grabens of Malawi. The BFSs of the Usisya and Metangula half-grabens are only slightly offset from each other and the BFSs of the Livingstone and Usisya units virtually merge end-to-end (Fig. 3). One variation on this general theme is for half-grabens to face each other imperfectly, like a pair of vertically skewed parentheses (this is exemplified by the North and East Kigoma half-grabens of Tanganyika, Usisya and the

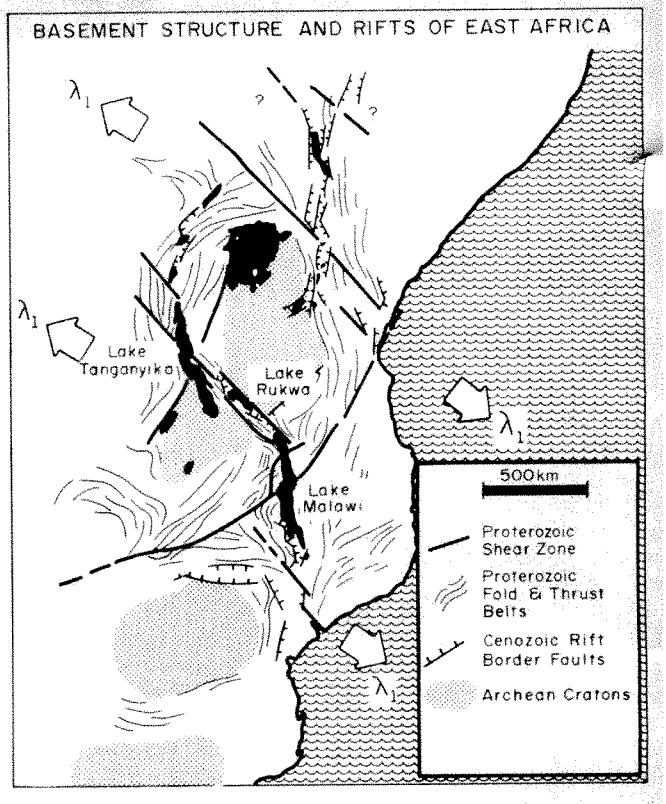


Fig. 1 Tectonic framework of East Africa.

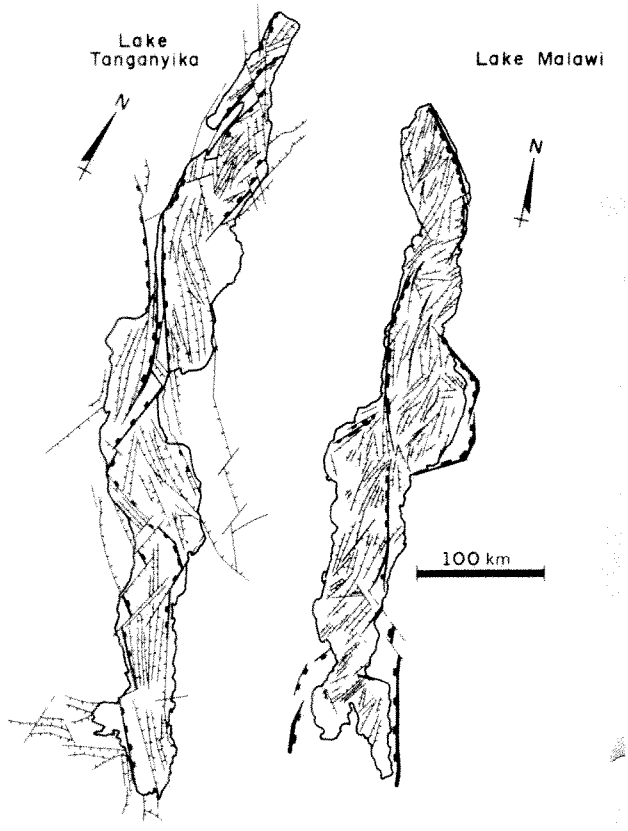


Fig. 2 Detailed fault-pattern maps of the Tanganyika and Malawi Rift Zones, derived from analyses of multifold seismic data. Faults shown are those which offset acoustic basement. Heavy hachured faults refer to master border-fault complexes.

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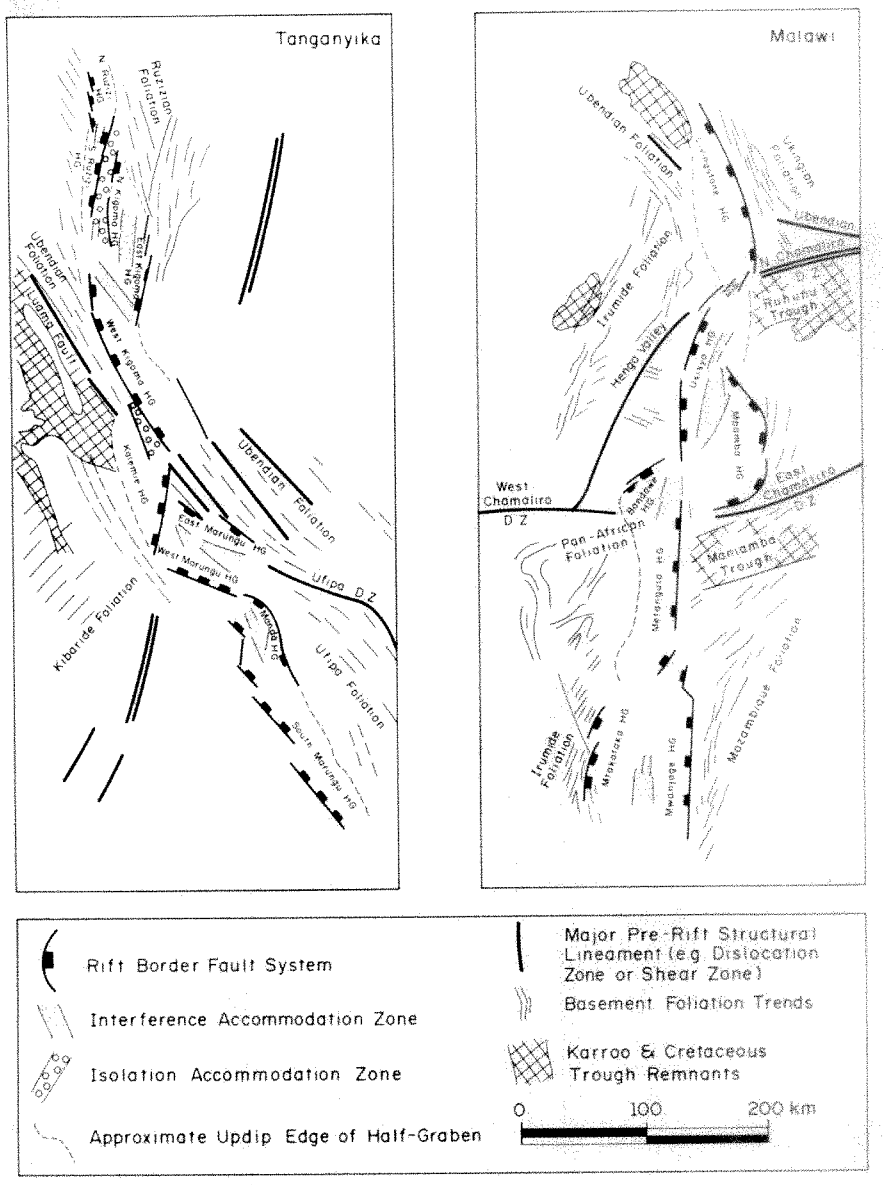


Fig. 3 Generalized structure maps of the Tanganyika and Malawi Rift Zones superimposed on pre-rift structure. The Tanganyika fabric is that described by McConnell and shown in the UNESCO maps of Africa[1-3,41,42], supplemented with more recent mapping and satellite image analyses[12]. The Malawi fabric is derived from Bloomfield[43] and an analysis by Versfelt[12].

Mbamba half-graben of Malawi). The resulting linking structure is called an interference accommodation zone (INAZ)[11]. Tanganyika has about five such zones, and Malawi has four (Fig. 3). INAZs are usually basement highs along which deformation is concentrated. The exact origin of INAZs is uncertain but their structural relief and tendency to act as strain guides suggest that they express a space limitation, or competition for space, associated with the facing geometries[8,11]. Another linking arrangement is for half-grabens to face in opposite directions, like a pair of reversed parentheses, with a backbone of relatively unsubsided terrain separating the half-grabens[4-8]. This linking structure is termed an isolation accommodation zone (ISAZ)[11]. Two ISAZs are mapped in Tanganyika, but there are none in Malawi.

The pre-rift structure of East Africa can be divided into the following categories: cratons and mobile belts, earlier rift troughs, transcontinental dislocation zones and basement foliations[12]. Cratons are thick, stable, Archaean nuclei composed of greenstone belts sandwiched between ensialic plutons. Mobile belts are gently arcuate, highly folded Proterozoic rocks that wrap around cratons[24]. Earlier rift troughs refer to remanents of Permo-Triassic- to Cretaceous-age rift basins. The Ruhuhu and Maniamba Karroo troughs in Malawi trend nearly perpendicular to the lake axis, whereas the Cretaceous trough at the north end of the lake runs essentially parallel to the lake. In Tanganyika, a Permo-Triassic basin trends sub-parallel to the southern half of the lake and intersects the lake near the central dog-leg (Fig. 3). Transcontinental dislocation zones (TDZs) refer to major sutures in the pre-rift crust that have been activated during various African orogenies and rifting episodes. They generally lie within mobile belts. The most important TDZs in Malawi are the West, East and North Chamaliro zones and the Henga Valley zone, all of which constitute local segments of the Schliesen–Mwimbeshi–Chamaliro TDZ (Figs 1 and 3)[25,26]. This TDZ may have represented a dextral shear zone during the Pan-African orogeny[25], and a sinistral strike-slip fault zone during Karroo times[26]. The most important TDZ in Tanganyika is the northwest-trending Rukwa dislocation zone (Fig. 1), which connects with the Ufipa zone and Luama Fault (Fig. 3). This zone may have originated during the Ubendian orogeny as a sinistral shear zone[1,27] and have been reactivated during Permo-Triassic rifting[27-29]. Basement foliation refers to the overall fabric of the pre-rift rocks and includes local faults and fractures, axial planar fabrics, trends of igneous bodies, contrasts in metamorphic grades, schistocity and compositional layer boundaries. Generally, this foliation is the local fabric of the mobile belts in which Tanganyika and Malawi are situated.

Estimates of the regional direction of extension in the Tanganyika–Rukwa–Malawi system range from northwest–southeast through southwest–northeast, but the evidence for

northwest–southeast opening is overwhelming. This extension direction is supported by numerous earthquake studies (refs 30–35 and R. M. Hamilton, unpublished report) and mapping and satellite image analyses (J. Chorowicz, preprint)[12,26–29,38–43]. Such an opening direction is essentially parallel to the Rukwa, Aswa and Zambesi lineaments, all of which presumably would serve as transform faults[11,27–29] with continued northwest–southeast opening.

The inter-relationships between rift architecture and pre-rift structure in Tanganyika and Malawi are shown in Fig. 3. The key relationships are summarized in Fig. 4. The most significant relationship is that alternations in half-graben polarity tend to occur where the transcontinental dislocation zones intersect the rift zones. In Malawi the switch in dip asymmetry between the Livingstone and Usisya half-grabens occurs where the Henga Valley–North Chamaliro TDZs cross the rift zone. The polarity switch between the Usisya and Metangula half-grabens occurs where the West Chamaliro–East Chamaliro zone crosses the rift. In Tanganyika the polarity reversal between the West Kigoma and Kalemie half-grabens occurs where the Rukwa–Ufipa–Luama TDZ intersects the rift[9,10,15]. All three of these intersections and polarity changes are associated with major kinks in the rift zones.

Not all alternations in half-graben polarity are associated with known TDZs, although all known TDZs are associated with alternations. For example, the reversal between the North and South Ruzizi half-grabens in northern Tanganyika (Fig. 3) does not correlate with any known pre-rift dislocation zone. Whether TDZs cause adjacent half-grabens to alternate polarities or whether they simply offer a convenient place for such switch-overs to occur is not certain. We favour the mechanical convenience hypothesis, but readily acknowledge that TDZs could fundamentally alter rift dynamics under certain conditions.

The orientation of a TDZ relative to the rift axis may be related to the type of linking structure observed. The Henga Valley–North Chamaliro dislocation zone in northern Malawi is oriented nearly perpendicular to the inferred extension direction, whereas the Rukwa–Ufipa–Luama TDZ in Tanganyika is essentially parallel. In the latter case, the border fault systems of the adjacent half-grabens splay off the TDZ[9,10,15] and become more orthogonal to the direction of extension with increasing distance from the TDZ (Fig. 4). In the northern Malawi example, the BFSs also peel off the TDZ, but become more parallel to the extension direction with increasing distance from the TDZ. In the central Malawi case, the Usisya and Metangula BFSs essentially die out as they approach the TDZ at right angles. Here, the extension direction is oblique to both the BFS and the TDZ. These differences may explain the different linking structures observed between the Tanganyika case (ISAZ), the northern Malawi example (very small, narrow INAZ) and the central Malawi situation (broad, diffuse INAZ). Admittedly, the morphology of the latter may also be partly due to the facing geometry of the Bandawe–Mbamba half-graben.

The role of earlier episodes of rifting on the development of the Tanganyika and Malawi Rifts is difficult to isolate from that of the TDZs because there is a close spatial association between these features. Evaluation of existing data suggests, however, that the rift architectures shown in Fig. 3 would not be significantly different if the earlier rift troughs were not present.

As stated earlier, both the Tanganyika and Malawi Rift Zones generally follow the basic 'grain' of the mobile belts. More surprising is the close association of INAZs with pre-rift fabric (Figs 2 and 3). This relationship is not sensitive to INAZ fault orientations relative to extension direction. Apparently, the fabric of the Proterozoic mobile belts exerts a very strong influence on the orientation of the structures that define INAZs. Careful inspection of Fig. 3 shows that INAZs occur commonly at the intersections between pre-rift fabrics of different trends. The INAZs that divide the North, East and West Kigoma half-grabens of Tanganyika are an example. In effect, INAZs divide different basement lithologies and follow the orientation of one of them. This may offer a clue to the origin of INAZs and perhaps also to the factors determining the geometries of facing half-graben.

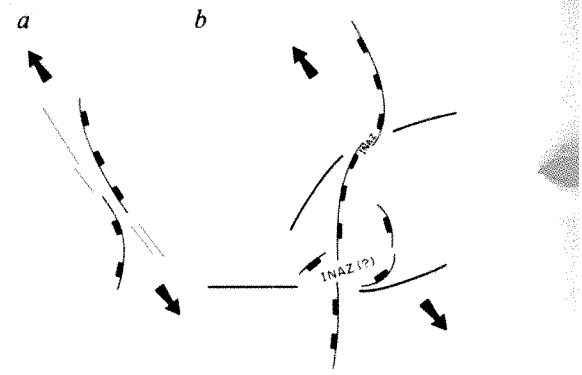


Fig. 4 Selected linking architectures in the Tanganyika (*a*) and Malawi (*b*) Rift Zones superimposed on pre-rift structure. Arrows denote inferred direction of extension for the East African Rift. *a*, Kavala Island model of ISAZ-linked half-graben alternation; the pre-rift shear zones and/or fabric are oriented sub-parallel to the primary extension direction. *b*, Malawi model of INAZ-linked half-graben alternation; the pre-rift shear zones and/or fabric are oriented sub-perpendicular to the primary extension direction.

There also seems to be a general parallelism between the trends of border-fault systems, internal faults and the pre-rift grain, with a few exceptions. One exception occurs where BFSs splay off TDZs and cross pre-rift fabric, as is the case for the West Kigoma and Kalemie BFSs. Another exception is the north–south linear portion of the Usisya–Metangula BFS pair, which seems to cut across the earlier grain. A third exception occurs in the Livingstone half-graben of northern Malawi, where the infrastructure tends northeast–southwest, at about 60° to the trend of the BFS and to the Ubendian and Ukingian foliations (Figs 2 and 3). In spite of the broad correlation between the trends of BFSs and pre-rift fabric, BFSs do not seem to follow major lithological contracts for appreciable distances[18]. This role seems to be reserved for INAZs.

The architectural concepts described for the Tanganyika and Malawi Rift Zones seem to have wide applicability[4,11], suggesting that the relationships described herein probably apply elsewhere. We speculate that the polarity reversals that occur in such rifted environments as the Gulf of Suez, Rio Grande Rift and Mid-Continent Rift of North America[4] are related to the positioning of transcontinental dislocation zones; we hope that this hypothesis will be tested in the future.

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Facultative viviparity in a thrips

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The absence of species that show facultative variation in reproductive mode within populations[1–4] has thus far severely hindered study of the selective pressures associated with oviparity and viviparity. Here I report that females of the mycophagous thrips *Elaphrothrips tuberculatus* (Insecta: Thysanoptera) are facultatively viviparous and produce male offspring by viviparity and female offspring by oviparity. Individual females can switch between the two reproductive modes. Females produce fewer offspring when viviparous than when oviparous, but the survivorship of viviparous offspring is sufficiently high that the average number of pupal offspring produced by females in each mode is similar. Facultatively viviparous thrips provide the first experimental systems for analysing the behavioural, ecological and physiological causes of variation in reproductive mode.

Elaphrothrips tuberculatus is a winged thrips about 5 mm long that lives on clusters of hanging dead oak leaves and feeds on fungal spores[5,6]. In southern Michigan, it overwinters as adults of both sexes in leaf litter, emerges in mid-April to mid-May, and undergoes two generations per year. Observations of breeding adults show that some females deposit eggs one at a time, over a 1–3-week period, onto an egg mass which they defend against potential egg predators[7]. By contrast, other females do not lay eggs, although they are gravid during any given breeding period. Dissection of gravid females demonstrates that oviparous females contain four ovarioles with each ovariole comprising enlarging, undeveloped oocytes arranged linearly. Viviparous females contain two clusters of embryos in increasingly advanced stages of development distally, such that fully gravid females contain one or several fully developed first-instar larvae. Two lines of evidence indicate that this variation among breeding females represents a reproductive dimorphism rather than stages of egg retention: first, the ovaries of more than 738 dissected females were of one or the other type, and second, gravid females isolated in Petri dishes deposit either a mass of undeveloped eggs that they guard ($N = 20$) or several first-instar larvae (with no trace of chorion visible) ($N = 53$). Larvae or eggs are often deposited within 24 h of isolation.

If reproductive mode is under facultative female control, then individual females may be capable of switching between oviparity and viviparity. *E. tuberculatus* females normally reproduce once during the one-month spring generation, but the summer generation lasts for about two months, which allows sufficient time for multiple bouts of reproduction[6]. Allowing females to breed individually in summer on dead oak leaves in nylon bags so as to record their reproductive mode (by noting the presence of egg masses and the presence and sex of offspring[6,8]) showed that about one-third of females switched reproductive mode in 1986 (21 both modes, 15 viviparous, 20 oviparous) and 1987 (20 both modes, 19 viviparous, 12 oviparous). *E. tuberculatus* is apparently the only animal species known in which individual females are capable of switching between oviparity and viviparity.

Because reproductive mode is under facultative female control in this species, variation in mode may be associated with variation in measurable aspects of females and their environments. The body sizes (lengths of the fore-femora) of viviparous and oviparous females did not differ during the spring generation, but during the summer generation viviparous females were substantially larger than oviparous females[9]. To determine whether or not aspects of seasonality affect female reproductive mode, I allowed individual females from both the spring and summer generation to breed on both spring- and summer-generation dead oak leaves (summer leaves have died within the past few months, whereas spring leaves died in the preceding year). The only treatment in which females were viviparous (5 of 14) was summer females on summer leaves; no females were viviparous in the spring females/spring leaves treatment (0 of 15), the spring females/summer leaves treatment (0 of 13), or the summer females/spring leaves treatment (0 of 14) ($G = 15.4$, $P < 0.005$). These data suggest that a proximate cause of viviparous reproduction involves interaction between a female's generation and the generation of the leaves that she inhabits.

All thrips are believed to have a haplodiploid genetic system, such that haploid males develop parthenogenetically, and diploid females develop from fertilized eggs[10,11]. Sexing of first-instar larvae[6,8] showed that, in *E. tuberculatus*, all of 1,051 newly hatched larvae from eggs laid by 42 females were female, and all of 202 larvae produced by 32 viviparous females were male. These data indicate that mode of reproduction is invariably associated with offspring sex. But sperm were seen clearly in the spermathecae of 134 (87%) of 154 dissected viviparous females.

Because females produce single-sex broods, individual breeding females can be considered as units of investment in one sex or the other[6]. Thus, if females are equal in resources available for breeding, then frequency-dependent selection on sex allocation[12] should favour a population-wide proportion viviparity (that is, a sex-allocation ratio) of 50%. In *E. tuberculatus*, deviations from 50% viviparity (Table 1) may be caused by a sex difference in the degree of competition among siblings between spring and summer[6] and partial bivoltinism with variation between generations in the reproductive value of the two sexes[13]. Moreover, if the large size of viviparous females relative to oviparous females in summer represents a difference in resources available for investment, then the summer sex-allocation ratio is more male biassed than it would appear to be from the proportion of females in each reproductive mode.

The association between offspring sex and reproductive mode in *E. tuberculatus* allows testing of a central hypothesis concerning the evolution of viviparity: that viviparous females should produce fewer offspring than oviparous females, but that these

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Table 1 Fecundity of oviparous and viviparous females and the relative survivorship of their offspring

Season	Pupal sex ratio	Mode	Percentage females in mode	Average fecundity	Relative survivorship
Spring	0.19	Viviparity	0.25	21.5	1.4
		Oviparity	0.75	43.7	1.0
Summer	0.58	Viviparity	0.55	13.5	2.1
		Oviparity	0.45	26.1	1.0

Within each sex, the number of pupae present at the end of a generation should be equal to the number of females in the appropriate reproductive mode during that breeding period, multiplied by their average fecundity, multiplied by the survivorship of their offspring from egg laying or larviposition to the pupal stage. Pupal sex ratio was calculated by collecting pupae during the spring and summer generations, allowing them to become adult, and sexing the adults (see refs 6, 9). The spring pupal sex ratio is the average for 1984 (0.19 male, $N = 9,709$), 1985 (0.16 male, $N = 6,617$), and 1986 (0.21 male, $N = 3,158$), and the summer pupal sex ratio is the average for 1985 (0.69 male, $N = 483$) and 1986 (0.46 male, $N = 1,399$) (ref. 6). The percentage of females in each mode was determined by dissecting females and inspecting their ovaries (see refs 6, 9); the percentage for spring is the average for 1986 (0.26 viviparous, $N = 449$) and 1987 (0.24 viviparous, $N = 902$), and the percentage for summer is data from 1986 ($N = 645$). Oviparous female fecundity was measured in spring by counting the number of eggs in complete clutches and taking the average for 1985 (39.5 ± 8.7, $N = 127$), 1986 (46.6 ± 9.4, $N = 121$), and 1987 (44.9 ± 9.9, $N = 145$) (refs 6, 7). In summer, oviparous female fecundity was measured by counting the number of eggs in completed and abandoned egg masses (26.1 ± 11.4, $N = 222$, data from 1986) (refs 6, 7). In spring, viviparous female fecundity was estimated by counting the number of first-instar larvae produced by individual viviparous females on 2–3 dead oak leaves in the field, dissecting the females near the end of the breeding period, counting the number of embryos in their abdomens, and adding these two numbers; these data (21.5 ± 5.4 offspring, $N = 20$ females) were taken in 1987 (ref. 6). In summer, viviparous female fecundity was measured by putting females individually on 2–3 dead oak leaves in nylon bags suspended in the field early in summer, allowing them to breed for about 2 months, and either: (1) counting the number of adult males that were produced by the females that were viviparous (15.0 ± 6.7, $N = 15$, data from 1986), or (2) removing and counting the larvae produced every two weeks for eight weeks (12.2 ± 6.9, $N = 18$, data from 1987) (ref. 6). The value used is the average for 1986 and 1987 (13.5 ± 6.8, $N = 33$). The relative survivorship, from the egg stage to the pupal stage, of offspring produced by oviparity is set arbitrarily to 1.0, and the relative survivorship of offspring produced by viviparity is obtained by multiplying through.

offspring should have higher survivorship[14]. In both generations, the average fecundity of viviparous females was about half that of oviparous females, but the survivorship of the offspring of viviparous females to the pupal stage was 1.4 times higher than that of the offspring of oviparous females in spring and 2.1 times higher in summer (Table 1). The contribution of offspring sex, rather than mode of development, to differences in survivorship is unknown. This hypothesis could be tested more directly using a thrips species, such as *Sporothrips amplus*, in which one sex (males) is produced by both oviparity and viviparity (unpublished data).

In vertebrates, correlates of viviparity include the presence of maternal care in related oviparous species[3,4] (which is considered as an alternative adaptation to harsh conditions for eggs[15]) and the presence of physical defences such as venom or predatory habits (which are presumed to reduce mortality in females encumbered by embryos[4,16]). In *E. tuberculatus*, maternal care is clearly an alternative to viviparity, and females use a potent defensive secretion, juglone, to repel predators such as salticid spiders (Blum *et al.*, unpublished data). Thrips apparently show a complete range of variation in reproductive mode, including fully oviparous, egg-retaining, facultatively viviparous, and fully viviparous species[17–19]. Comparative analysis of the correlates of reproductive mode among thrips, coupled with experimental analysis of facultatively viviparous species, should elucidate the selective pressures associated with the two modes of reproduction. Moreover, the presence of facultative viviparity in this taxon suggests that viviparity sometimes originates as a facultative alternative in an otherwise oviparous population, rather than by population-wide directional selection for egg retention.

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A mutation that changes cell movement and cell fate in the zebrafish embryo

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The study of developmental patterning has been facilitated by the availability of mutations that produce changes in cell fate, in animals such as *Caenorhabditis elegans*[1,2] and *Drosophila melanogaster*[3,4]. We now describe a zygotic lethal mutation in the zebrafish, *Brachydanio rerio*, that also changes how particular embryonic cells develop. Severe pattern deficiencies are observed that are restricted to a single body region, the trunk. The mutation may directly affect mesoderm, as somites do not form in the trunk. Head and tail structures, including tail somites, are relatively undisturbed. The earliest detected expression of the mutation is during gastrulation, when movements of mesodermal cells occur incorrectly. We injected prospective trunk mesodermal cells with lineage tracer dye and observed that in mutants these cells may enter a new body region, the tail, and there may express a new fate appropriate for the changed position.

The mutation, *spt-1(b104r1)*, nicknamed 'spadetail', was initially discovered among the parthenogenetic progeny of a

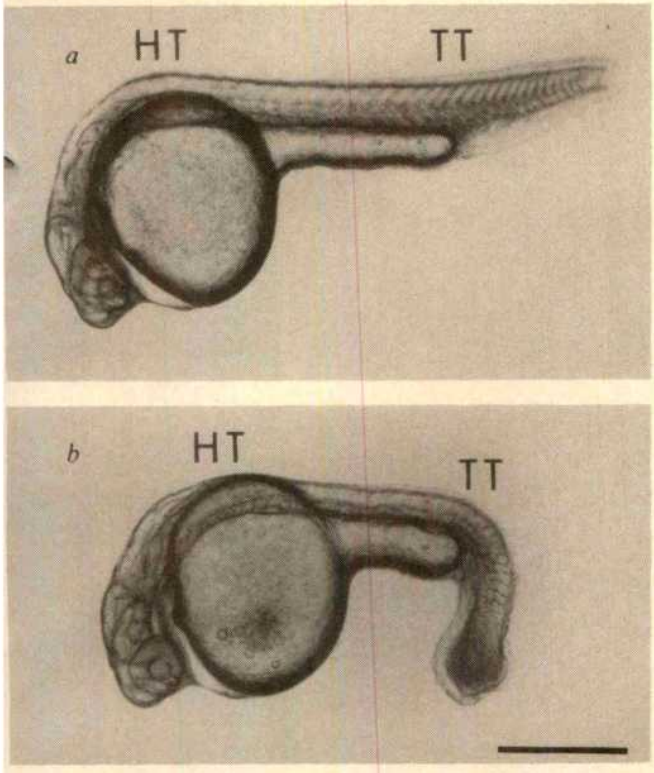


Fig. 1 Live wild-type (*a*) and homozygous *spt-1*(*b104*) mutant (*b*) zebrafish embryos at 24 h (all times are in hours postfertilization at 28.5 °C). Genetic nomenclature for zebrafish is adapted from that for *C. elegans*[17]; *spt-1* indicates the locus of the mutation, and *b104* is the allele. HT: region of transition between the head and trunk, at the level where the anterior-most somites are normally observed. These somites are missing in the mutant, and trunk paraxial (segmental plate) mesoderm is deficient. TT: region of transition of the trunk and tail, at the anus, that in the mutant marks the position of a severe kink of the body and the restoration of somites. The enlarged tail bud of the mutant contains cells that eventually degenerate. (The designation *spt* refers to this prominent phenotype, termed by us the '*sp*ade*t*ail'). Scale bar, 400 μm.

single heterozygous female. The origin of the mutation is unknown. Mutants were identified by the changes in appearance of the embryo shown in Fig. 1. The phenotype is zygotic recessive, and mutants survive for less than one week of development. The mutation segregates as a single locus, and is fully penetrant; about one-quarter (410/1,660) of embryos obtained from crosses between heterozygotes were mutant, and about one-half (222/437) of haploid embryos obtained[5] from heterozygous females were mutant. As a step towards characterizing the locus genetically, we have recently identified a putative allelic γ-ray-induced mutation, *b141*, by its failure to complement the original one, *b104*. We have not yet studied the phenotype of *b141* homozygotes however, and describe here the *b104* mutation only. We concentrate on the earlier changes, which are likely to be more useful for our eventual understanding of *spt-1*+ function.

Development seems normal before gastrulation, and during its onset. By six h (hours after fertilization at 28.5 °C) both wild-type embryos and *spt-1* mutants have begun epiboly[6], and have formed a germ ring and embryonic shield[7]. The presence of these structures suggest that early morphogenetic cell movements occur in mutants, in particular the involution of mesoderm (ref. 8; R.M.W. and C.B.K., manuscript in preparation). After 7–8 h cells accumulate abnormally at the dorsal side of the gastrula to produce a transient swelling, whose anterior–posterior level corresponds approximately to the region of transition between the prospective head and trunk (HT; Fig. 2*a*, *b*). As epiboly is completed at 10 h, cells accumulate at a

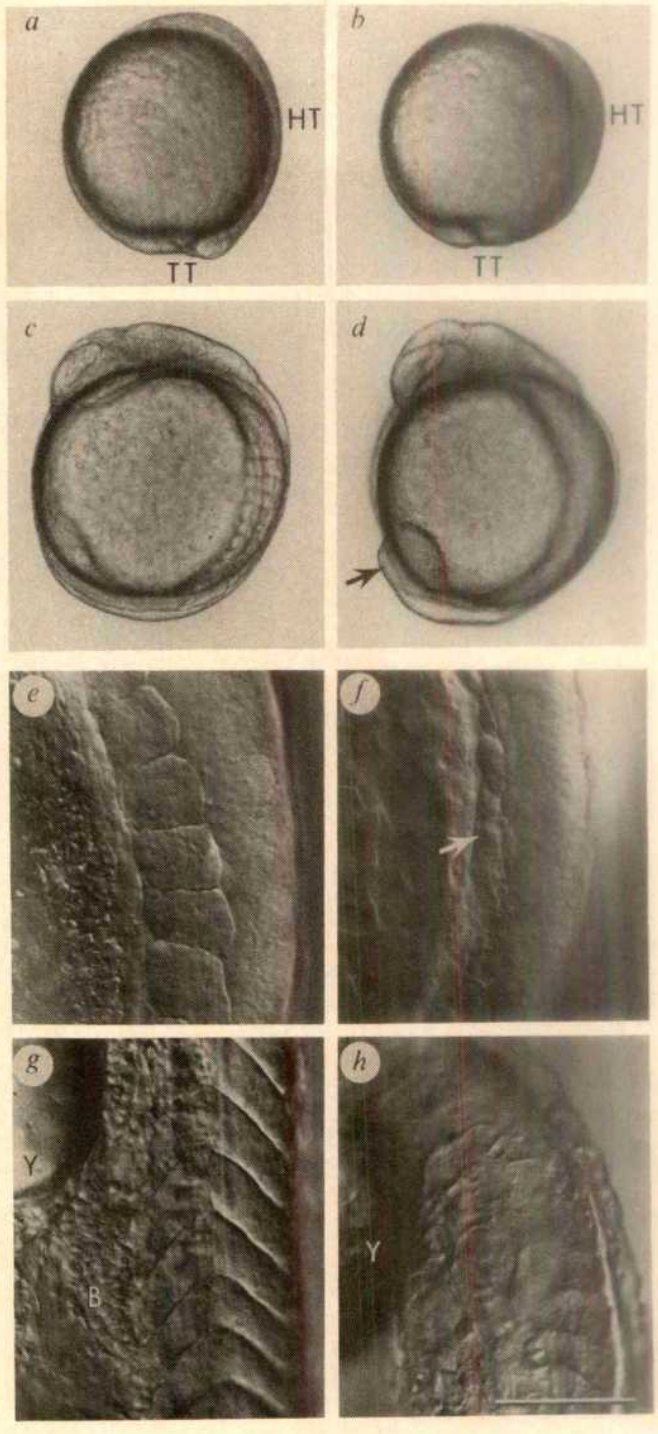


Fig. 2 Morphogenesis in living wild-type (left panels) and *spt-1* mutant embryos (right panels). Left side views with anterior to the top. *a*, *b*, Wild-types and mutants at completion of epiboly (closure of the yolk plug) at 10 h. The HT swelling was first observed in this mutant (*b*) at 7 h and the TT swelling has just appeared. *c*, *d*, Wild-type and mutant early tail bud embryos at 13.5 h. Trunk somites are visible in the wild-type (*c*), but are not present in the mutant (*d*), which by this stage has developed a very prominent tail bud (arrow). *e*, *f*, Nomarski interference contrast views of the developing trunk at 13.5 h. Somites are well-formed in the wild-type (*e*). The mutant (*f*) paraxial mesoderm (arrow) is very deficient; most of the tissue present is the spinal cord. *g*, *h*, Nomarski views of the TT region at 23 h. In the wild-type (*g*), V-shaped myotomes are present in both the trunk and tail. The anus (present but not visible at this plane of focus) is located just posterior to the yolk sac (Y), and near this location is a prominent cluster of developing blood cells (B). In the mutant (*h*), the first somites to appear are located over the posterior yolk sac. The myotomes that develop from them are poorly formed, whereas more posterior myotomes in the tail are better formed. The anus and blood cells are absent. Scale bars, 400 μm (*a*–*d*); 100 μm (*e*–*h*).

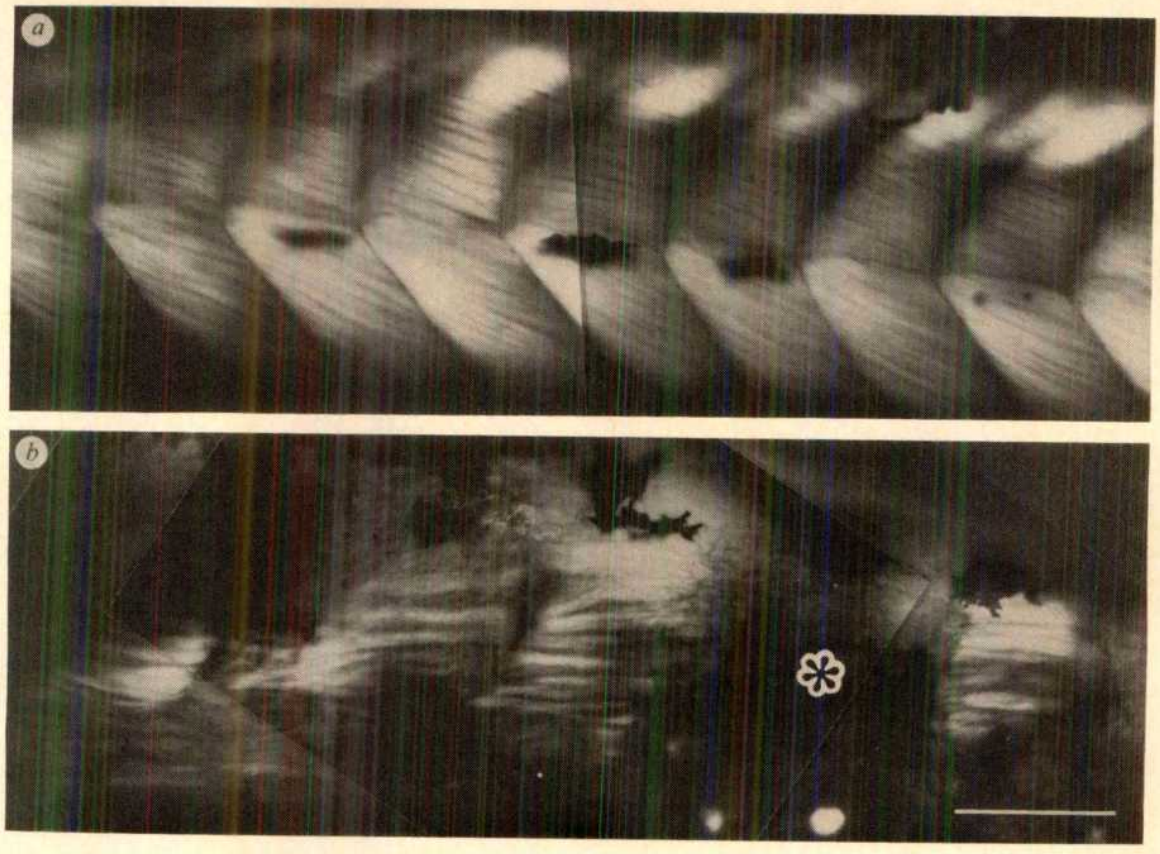


Fig. 3 Imperfect myotomes appear in the trunk in *spt-1* mutants. Left side views (dorsal to the top) of trunk segments in live embryos at 72 h. Polarized light, revealing patterning of birefringent myotomal muscle fibres. In wild-type embryos (*a*) the myotomes are regular V-shaped structures, and contain muscle fibres in orderly obliquely longitudinal orientations. The transverse myosepta (separating myotomes) and the horizontal myosepta (separating dorsal ventral parts of the myotomes) do not contain muscle, and are not birefringent. The view of a mutant (*b*) shows anterior trunk myotomes. The myotomes are of approximately normal length, and some muscle fibres are oriented longitudinally. Mutant trunk myotomes are not V-shaped, and horizontal myosepta are not detected. There are fewer muscle fibres than normal. Here, three anterior myotomes are followed by a myotome-long space with no muscle (*), and then by a single (isolated) myotome with severely disorganized fibres. Wild-type embryos normally have 15 myotomes anterior to the anus at this stage; this mutant had 6. Scale bar, 100 μm.

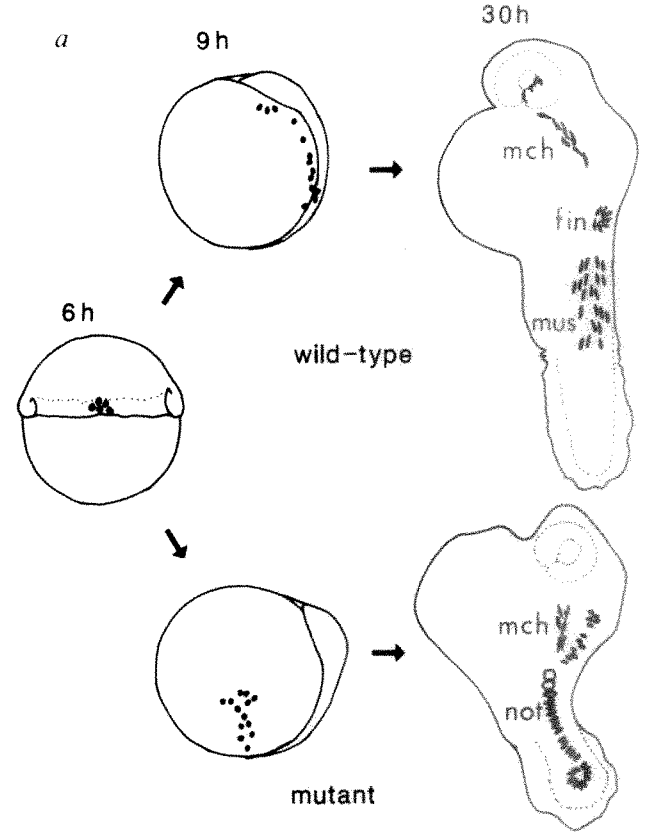
second incorrect location, ventral to the location of yolk plug closure, and approximately at the region of the juncture of the trunk and the tail (TT; Fig. 2*a*, *b*). The cells in this second swelling then seem to enter the tail bud, which remains abnormally large as the tail develops (Fig. 2*d*, arrow).

During tail bud stages, paraxial mesoderm is depleted in the trunk region of *spt-1* embryos (Fig. 2*c–f*). As trunk somites are forming in wild-type embryos (11–16 h; Fig. 2*e*), there are only scattered mesenchymal cells, and a few clusters of cells adjacent to the notochord in the mutants (Fig. 2*f*, arrow). The notochord and overlying spinal cord of the trunk at first seem to be formed normally, and then develop bends and kinks, as if they were compressed along the anterior–posterior axis, with the most severe kinks near both the HT and TT. Later some mutants fail to develop structures that normally arise near the HT, including the pronephric kidneys and pectoral fins on one or both sides. Blood cells and the anus, both normally arising near the TT, may also be missing. Structures that lie anterior to the trunk are invariably present however, and develop fairly normally, for example, the brain (including hindbrain neuromeres[9]), head sense organs, the series of branchial arches, the heart, and the pericardial hatching gland.

At the posterior in *spt-1* mutants, beginning at about the TT, paraxial mesoderm is more abundant and somite furrows are observed, roughly on schedule (15–16 h) with the formation of tail somites in their wild-type sibs (Fig. 2*g*, *h*). The earliest somites to form in mutants, those near the TT, are poorly shaped at first, but the shape of these somites is better at later times, and the posterior-most somites form rather normally at the outset. Eventually tail myotomes, derived from them, appear normal.

None of these changes are accompanied by an unusual amount of cell death. The only extensive cell death that we observe during embryogenesis in mutants is in the enlarged tail bud, beginning after the end of somitogenesis at about 30 h. First, a few necrotic cells can be identified in the tail bud with Nomarski optics[10], and by 48 h degeneration is massive. As a consequence, the abnormally swollen tail bud (Fig. 1) eventually disappears. The cell death in the mutant tail bud might be an entirely normal morphogenetic process that is magnified by its excessive number of cells. We have observed some corresponding necrosis in wild-type embryos, and similar patterns of cell death have been observed after completion of segmentation in the chicken[11] and leech[12].

We interpret the phenotype to mean that the mutation most severely affects development of the trunk of the body. The trunk does not seem to be deleted altogether as, for example, in *Drosophilia* gap mutants[13]. Some trunk structures, such as the notochord and the spinal cord, develop on a wild-type schedule. Moreover, a particularly interesting finding is that, later on, pattern regulation occurs in the trunk. Thus, before 24 h, myotomes become evident in this body region; that is, where no somites were present initially (Fig. 3). We have not determined the cellular origin of these myotomes. They are of about the correct axial length, but are not V-shaped as normal, and are sometimes separated from each other by myotome-long



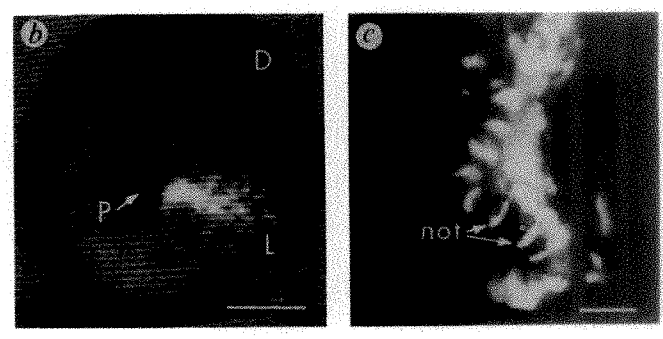


Fig. 4 *a*, Cell movements and fates of prospective trunk somitic mesoderm are changed in *spt-1* mutants. Single clones of fluorescently labelled cells that occupied the lateral germ ring at 6 h (early gastrula) were followed in wild-type (top) and mutant (bottom) embryos. The drawings were made from video time-lapse sequences of images (as in *b*), and show left-side views with anterior to the top. In the wild type the cells converge dorsally by 9 h, and by 30 h have formed mesoderm of the head and trunk, including mesenchyme (mch), pectoral fin bud (fin) and somitic muscle (mus). In the mutant, the cells moved posteriorly to populate the caudal trunk and the tail. They formed mesenchyme, notochord (not) and mutant tail bud. The clones in both the wild type and mutant also included neural and surface epithelial derivatives, which are not shown in these drawings. *b*, A posterior view of this mutant at 9 h, photographed from the face of the video-monitor. Fluorescence due to the tracer is superimposed on the bright-field image. The labelled cells have formed a stream from their starting position in the lateral equatorial region (L) towards the yolk plug posteriorly (P). In wild-type embryos, cells at the same starting location move dorsally (D). *c*, Fluorescence video micrograph of the tail (side view) of the same mutant at 30 h. Differentiating notochord cells (not) are labelled, distinguishable by their position and distinctive disk-shape. The individual cells are separated from one another by unlabelled notochord cells, among which they have intercalated earlier in development; the intercalations are a normal feature of morphogenesis[15]. In wild-type diploid embryos (as in *Xenopus laevis*[18]) the notochord of the head, trunk, and tail derives from cells located adjacent to the dorsal blastoderm margin of the early gastrula (C.B.K. and R.M.W., manuscript in preparation). In the work presented here we observed that dorsal marginal cells also gave rise to notochord in both mutant and wild-type haploid embryos (three out of five clones in mutants and three of 10 clones in wild types; at the gastrula stage these clones were all located along the dorsal one-third of the germ ring). Lateral and ventral marginal cells frequently gave rise to notochord in haploid mutants (five of 12 clones that were located along the lateral and ventral two-thirds of the germ ring in the gastrula), but not in wild types (none out of seven lateral and ventral clones). A more complete description of the mutant fate map is in preparation.

Methods. As mutants could not be identified until after the beginning of these experiments, we obtained a high proportion (50%) of mutants, by using haploid progeny[5] from *spt-1/+* heterozygotes. The mutant phenotype in haploids is similar to, and generally more severe than, that of homozygous diploids. Single clones were marked in a series of embryos at about 3 h (about the 10th cleavage division), by injecting cells near the blastoderm margin with rhodamine-dextran[19]. The position of the clones was mapped at early gastrula stage (6 h) using a Silicon-Intensified-Target (SIT) video camera to amplify fluorescence. In some cases of embryos bearing lateral clones (positioned at 90° from the embryonic shield[7]) the movements of the labelled cells were recorded during the next nine hours by time-lapse video microscopy. At 30 h we determined whether the embryos were wild-type or mutant, and scored the positions and the differentiated fates of the labelled cells[7]. Scale bars, 200 μm (*b*); 50 μm (*c*).

spaces that contain no muscle (Fig. 3*b*, *). The correct number of myotomes (30 pairs, including the trunk and tail) is also not restored in *spt-1* mutants. Counts in mutants range upwards from about 14.

Many of the defects in *spt-1* mutants may arise secondarily, cascading from an early pattern disturbance that involves the movements of specific cells during gastrulation. The HT and TT mark both the early locations of the abnormal cell accumulations and the later regions of most severe pattern defects. Paraxial mesoderm (prospective somite) is deficient between these same two regions (that is, in the trunk). These results indicated that those cells which should have migrated in the gastrula to form trunk paraxial mesoderm, moved incorrectly, leaving the trunk cell-deficient. We examined this possibility directly by using intracellularly injected tracer dye to observe the movements and eventual fates of the mutant cells. Previous work[7,14] established that the trunk somitic mesoderm normally arises by the dorsalwards convergence of cells from the lateral part of the germ ring at early gastrula stage. These cell movements seem to be similar in the haploid wild-type embryos studied here, and cell fates include trunk somitic muscle (Fig. 4*a*, top). However, in haploid *spt-1* mutants (Fig. 4*a*, bottom) cells at the same location in the early gastrula fail to converge dorsally; rather they move posteriorly, in an apparently active fashion (Fig. 4*b*), to enter the tail. The derivatives of such dislocated cells often include unusual fates for cells from this beginning location, in particular the notochord (Fig. 4*c*). Notochord of both the trunk and tail is normally derived from the dorsal side of the early gastrula, not the lateral side (see Fig. 4 legend). Thus the *spt-1* mutation can change the fate, as well as the movements of mesodermal cells. On the other hand, transfating was not invariably observed; in some mutants the dislocated cells formed tail muscle.

From the early expression of the mutant phenotype we infer that *spt-1+* must normally function during (or before) gastrulation. Our studies have not revealed the primary gene function, or which cells express the gene. The earliest observed change is in cell movement, and perhaps the mutation directly perturbs movement—affecting either the mesodermal cells themselves or their environmental navigation cues. If so, the observed changes in pattern and cell fate could follow secondarily, because final cell positions are changed. Alternatively, the mutation could act more cryptically: if cell commitments occur during gastrulation[15], the mutation could alter those commitments, and the cells move improperly as a consequence. Study of embryos that are genetically mosaic[16] for *spt-1*, as well as determining the primary function of the gene, should elucidate our

understanding of the relationships of cell movement, position and fate during embryogenesis in this vertebrate.

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Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons

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Nerve growth factor (NGF) is a trophic molecule essential for the survival of sympathetic and sensory neurons during ontogeny[1]. The extent to which NGF is involved in the maintenance or regulation of the differentiated phenotypes of mature peripheral neurons is much less clear, however. Biochemical analysis of the actions of NGF upon peripheral neurons has been hampered by the lack of a preparation of neuronal cells that are responsive to NGF but do not require it for survival. We report here that in adult dorsal root ganglion neurons, which can be isolated, enriched and maintained in culture in the absence of neuronal growth factors[2], the expression of mRNAs encoding the precursors of two neuropeptides, substance P and calcitonin gene-related peptide is regulated by NGF. Our results provide the first direct evidence of a continuous dynamic role for NGF in regulation of peptide neurotransmitter/neuromodulator levels in mature sensory neurons.

When adult dorsal root ganglion (DRG) neurons were grown in culture in the absence of NGF, the level of mRNA encoding the substance P precursor (preprotachykinin, PPT) declined progressively over 5 days (Figs 1, 2*a*). The substance P content of parallel cultures (Fig. 2*b*) declined by 60-70% over the same period. In cultures maintained in the presence of NGF (25 ng ml^{-1}), levels of PPT mRNA did not differ significantly from controls for the first 24 h, rose 1.5-4-fold (range in six experiments) over the next 24-48 h and did not change significantly thereafter. The abundance of PPT mRNA was 4-6-fold greater in cultures exposed to NGF for 48 h, compared with those deprived of NGF. After 5 days, PPT mRNA levels were 15-60-fold higher in NGF-treated cultures than in untreated cultures, as much because of a decline in PPT mRNA in NGF-deprived cultures as a continuing increase in PPT mRNA levels in response to NGF. Over the time course of these studies, the substance P content of NGF-treated cultures rose by approximately 50%.

Most, if not all, of the subpopulation of rat DRG neurons which exhibit substance P-like immunoreactivity also contain

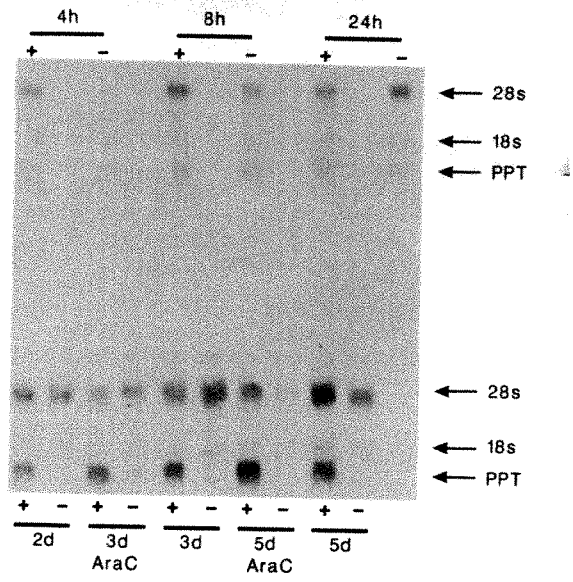


Fig. 1 Northern blot analysis of the effect of NGF upon the levels of PPT mRNA in adult rat DRG neurons. Replicate cultures of enriched adult DRG neurons (10,000 neurons per well) were established at $t = 0$. The mitotic inhibitor cytosine arabinoside (Ara C, 10^{-5} M) was present in replicates of the 3-day and 5-day cultures. At each time point, the neurons from four wells, grown in the presence or absence of NGF, were pooled, and total RNA was isolated. RNA samples were electrophoresed on formaldehyde-agarose gels and probed with a ^{32}P-labelled RNA probe complementary to human PPT mRNA. Under the washing conditions used (2 × SSPE/0.1% SDS at 65 °C), the autoradiographic signals due to the nonspecific binding of probe to 28S and 18S RNAs could be used to compare the amounts of RNA on each lane of the gel. Under more stringent conditions (0.1 × SSPE at 65 °C) the intensity of the signal due to PPT mRNA was undiminished, but the nonspecific binding of probe to RNA was abolished.
Methods. Cultures of adult rat DRG neurons were prepared as previously described[2]. Briefly, pooled DRG (40-48 per animal) from adult Sprague-Dawley rats (200-250 g) were cleaned of connective tissue and dissociated into a suspension of single cells by trituration after collagenase and trypsin treatment. After enrichment by separation of neurons from non-neuronal cells on the basis of their differential adhesion to a polyornithine substrate after overnight incubation, 10,000 neurons were plated out in each of the four wells (coated with polyornithine and laminin) of 35 mm-well tissue culture dishes (Greiner). The cells were maintained at 36.5 °C, in an atmosphere of 97% air: 3% CO_2 in F-14 culture medium (Imperial) supplemented with 10% horse serum (GIBCO-BRL), and, where indicated, cytosine arabinoside (10^{-5} M; Sigma). Highly purified 2.5 S mouse salivary gland NGF was added at 25 ng ml^{-1}. The medium was changed after 3 days where appropriate. For Northern analysis, RNA samples, each obtained from 40,000 neurons, were electrophoresed on 1.1% agarose gels containing formaldehyde, and transferred to nylon membranes (Biodyne A: Pall Ultrafine Filtration, Glen Cove, New York). A radiolabelled β-PPT cRNA probe was obtained by transcription, using [α-^{32}P]CTP and T7 RNA polymerase, of a fragment of human β-PPT cDNA (bases 174-417 of the published sequence[18] which had been subcloned into the Gem3 vector (Promega). The human cRNA probe was more than 95% homologous to the corresponding region of rat β-PPT mRNA[19].

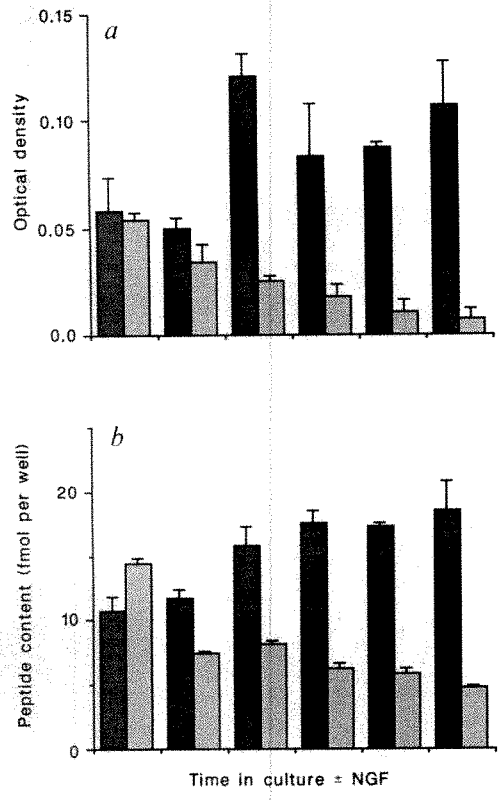


Fig. 2 Effect of NGF upon levels of (*a*) PPT mRNA and (*b*) substance P-like immunoreactivity in replicate cultures of adult rat DRG neurons maintained for the times shown in the presence (dark blocks) or absence (pale blocks) of NGF (25 ng ml^{-1}). **Methods.** *a*, Northern blots of total cellular RNA (derived from 40,000 neurons per sample) were hybridized with a PPT cRNA probe (see Fig. 1 legend) and results quantitated by densitometry using a Cambridge Instruments Quantimet 800 image analyser. *b*, The substance P-like immunoreactivity content of parallel cultures (5,000 neurons per sample) was determined by radioimmunoassay using a C-terminally directed, polyclonal substance P antiserum[20]. Values are mean ±s.e.m. ($n = 4$).

calcitonin gene-related peptide (CGRP)[3]. To establish whether the effects of NGF we described are specific for substance P or also affect the expression of other neuropeptides, we examined the levels of CGRP and its mRNA in the same or parallel cultures to those used above. As for substance P, the levels of CGRP mRNA declined (Fig. 3) in cultures deprived of NGF. CGRP mRNA levels were more than 15-fold higher in cultures treated with NGF for 5 days, than in similar cultures deprived of NGF. In parallel cultures in which CGRP content was measured by radioimmunoassay, a 4-fold increase in CGRP was detected over 4 days in culture in the presence of NGF (data not shown).

Although we have previously shown that the vast majority of adult rat DRG neurons survive in culture in the absence of NGF or other neurotrophic factors[2], we had to establish that the effects described above were not due to NGF promoting the survival of a small population of substance P-rich DRG neurons. Cultures were first deprived of NGF for 2 days (in total 3 days without NGF, including the enrichment procedure) and NGF was then added for 2 or 4 days, RNA was isolated, and PPT mRNA measured. Figure 4 shows that levels of PPT mRNA were elevated in NGF-treated cultures regardless of prior deprivation of NGF. We have shown previously that, even after NGF deprivation for 6 or more days, substance P levels in cultured adult DRG neurons can be elevated upon the addition of NGF[4]. We conclude, therefore, that the effect of NGF on PPT and CGRP mRNAs is not linked to survival but is due to a separate action of NGF on the function of adult sensory neurons.

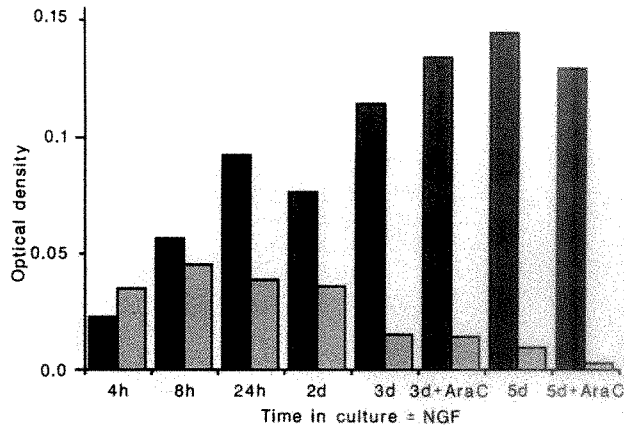


Fig. 3 Effect of NGF on the level of CGRP mRNA in duplicate cultures of adult rat DRG neurons mainained in the presence (dark blocks) or absence (pale blocks) of NGF (25 ng ml^{-1}) and, where shown, cytosine arabinoside (Ara C: 10^{-5} M). Northern blots of total cellular RNA (see Fig. 1 legend) were probed with a CGRP cRNA probe and the results quantitated by densitometry. The probe used was complementary to a 230-base pair sequence from 3′-untranslated region of human α-CGRP mRNA (ref. 21), ~76% homologous to the corresponding region of rat α-CGRP mRNA (ref. 22), and was generated using a commercial kit (Amersham).

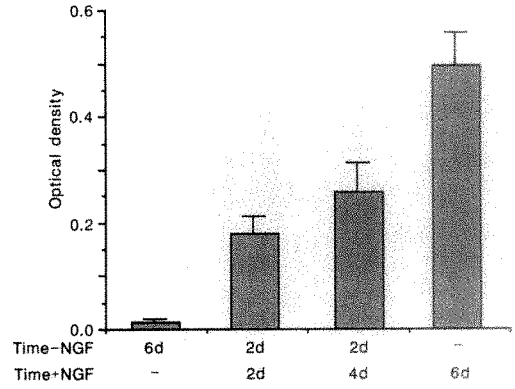


Fig. 4 Comparison of the relative levels of PPT RNA in replicate cultures of adult DRG neurons that were grown continuously in the presence of NGF, without NGF or in which the addition of NGF was delayed for 2 days after plating. Note that PPT mRNA levels were elevated (compared to controls) even after initial deprivation of NGF for 2 days. Values are mean ±s.e.m. ($n = 3$).

Although the procedures used to establish adult rat DRG neuronal cultures give an initial enrichment of >80% neurons, the proliferation of non-neuronal cells (Schwann cells and fibroblasts) cannot be suppressed without mitotic inhibitors such as cytosine arabinoside. To show that non-neuronal cells did not contribute directly or indirectly to the effects observed, several additional experiments were carried out. First, no PPT or CGRP mRNA was detectable in enriched cultures of non-neuronal cells grown in the presence or absence of NGF. Second, although cytosine arabinoside strongly inhibited the proliferation of non-neuronal cells in culture, this mitotic inhibitor did not influence the regulation of PPT and CGRP mRNA by NGF (Figs 1, 3).

There is now strong evidence that DRG neurons, once mature, do not depend on NGF for survival, as they do during development[5,6]. Instead, several lines of indirect evidence suggest that, in adult animals, NGF may be important in regulating the production of neuropeptides in sensory neurons. Various chemical, immunological and surgical interventions *in vivo* have been shown to lead to reductions in the levels of substance P

in adult DRG. For example, sciatic nerve section in the rat results in the loss of substance P from lumbar DRG, an effect attributed to NGF deprivation, as the substance P loss can be reversed by application of NGF to the cut stump of the transected nerve[7]. Given the considerable cell death in DRG after peripheral nerve section, it is possible[8] that the effects of exogenous NGF are related to protection against axotomy-induced cell death, rather than to direct stimulation of substance P biosynthesis. Further evidence that NGF may dynamically regulate the neuropeptide content of sensory neurons is provided by the observation that substance P levels are elevated in the target fields of sensory neurons after chemical (6-hydroxydopamine) or surgical destruction of NGF-dependent sympathetic neurons innervating the same structures; this elevation of substance P levels is probably due to an increased availability of target-derived NGF for the intact sensory neuron[9,10].

The observed differences in the levels of substance P or other peptides after NGF deprivation or administration *in vivo* are not evidence that NGF modulates the genes encoding the precursors of these peptides. Increased or decreased peptide levels could simply be a consequence of changes in the rates of translation, processing, release or degradation of the peptides themselves. The observed actions, the direct induction of the mRNAs, were not mediated indirectly through non-neuronal cells, nor were they linked to a survival-promoting effect of NGF. Our results suggest that a continuous supply of target-derived NGF is required as a retrograde effector to maintain the steady state levels of peptide neurotransmitters/neuromodulators found in mature sensory neurons. NGF treatment has been shown to evoke a number of rapid biochemical changes (including the phosphorylation of specific proteins[11–13] and the induction of certain genes[14–17]) in PC12 phaeochromocytoma cells. The system used here provides a means to establish the relevance of these observations to the mechanism of action of NGF in normal cells.

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Biosynthesis of the reverse transcriptase of hepatitis B viruses involves *de novo* translational initiation not ribosomal frameshifting

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Retroviruses and many other types of genetic elements replicate by reverse transcription of RNA (for reviews, see refs 1 and 2). Although structurally and biologically very diverse, such elements carry conserved polymerase genes (*pol*) that encode proteins required for reverse transcription[3]. In most cases, the *pol* gene is preceded by an overlapping gene encoding one or more nucleocapsid proteins[4], in a different reading frame. Because both coding regions are represented in a single mRNA, the question arises of how the reverse transcriptase in the alternative reading frame is expressed. In retroviruses and retrotransposons it is expressed as a nucleocapsid-polymerase fusion protein by ribosomal frameshifting during translation of the overlapping region[5–8]. We have examined the mechanism of polymerase biosynthesis in another family of animal viruses that use reverse transcription, the hepatitis B viruses. Genetic and biochemical studies reveal that these viruses do not use ribosomal frameshifting to generate this enzyme, but instead direct translation initiation at an internal initiation (AUG) codon in the polymerase gene.

The hepatitis B viruses (HBVs) are small DNA viruses that produce persistent hepatic infections in a variety of animal hosts and replicate their DNA genomes via reverse transcription of an RNA intermediate[9]. All members of this family contain an open reading frame (ORF), "P" (for *pol*), which is homologous

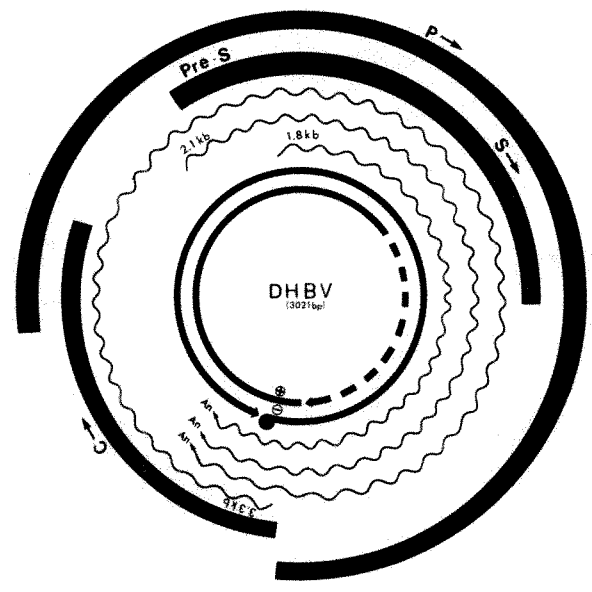


Fig. 1 Physical map of DHBV. Inner circles represent the DNA genome: the minus (−) strand (the strand synthesized through reverse transcription of viral RNA) is linked to a terminal protein[35] shown as a solid circle at its 5′ end; the plus strand is partially incomplete and shown by a dashed line at the 3′ end. The three wavy lines are the three main species of viral transcripts found in infected cells: the 2.1 kilobase (kb) mRNAs coding for the pre-surface (pre-S) antigens, the 1.8 kb mRNAs coding for the surface (S) antigens, and the 3.3 kb RNAs being the viral pregenome (the template for reverse transcriptase) and mRNAs coding for the core (C) antigens and viral polymerase (P). The three long open reading frames for *S*, *C* and *P* (*pol*) genes are drawn as filled boxes. Note that the 5′ end of the *P* gene is overlapped by the 3′ end of the *C* gene. For detail see ref. 36.

to retroviral *pol* genes. As Fig. 1 shows for the duck hepatitis B virus (DHBV)[10], the 5′ end of the P frame overlaps with the coding region (C) for the core (or nucleocapsid) protein; *pol* sequences are in the +1 frame relative to *core*. Figure 1 also shows the principal mRNAs identified in infected livers[11-14]. These include the subgenomic RNA species encoding the viral surface (S) proteins and the genome-length RNAs, one of which serves as the template for reverse transcription. As for other retroid elements, only the genome-length RNA contains the entire *pol* region. Such RNA is found in polyribosomes and is believed to be the mRNA for translation of both core and pol proteins. As no other (for example, spliced) viral RNAs have been reported in hepadnavirus infected cells, the main possibilities for polymerase biosynthesis are translational frameshifting to produce a core-pol polyprotein or translational initiation at an AUG in the *pol* gene. To date, no intact core-pol fusion proteins have been detected in productively infected cells (although truncated core-pol fusions were detected in some tumours in which viral DNA was probably rearranged[15]). However, pol-related proteins of relative molecular mass 70,000-90,000 (70-90K), without associated core determinants, have been identified in HBV virions[16,17]. It is unclear whether these polypeptides are the primary translation product of the *pol* gene or result from proteolytic cleavage of a large core-pol precursor.

To study pol biosynthesis we initially used a strategy which had been useful in the demonstration of translational frameshifting in retroviruses[5]. The *core-pol* overlap of ground squirrel hepatitis virus was cloned downstream of an SP6 bacteriophage promoter and *in vitro* transcripts were translated in extracts of rabbit reticulocytes, wheat germ, mouse liver and infected ground squirrel liver in attempts to identify a frameshift product. In all experiments, a detectable but low level (≤1%) of translational frameshifting occurred within the *core-pol* overlap (data not shown). Control experiments using the *pol-S* overlap (see Fig. 1) also exhibited this low level of frameshifting, suggesting that little or no specific frameshifting was occurring in the core-pol overlap region.

To assess the importance of the core-pol overlap *in vivo*, we constructed mutations in the *core* and *pol* genes designed to identify regions of the genome required for *pol* biosynthesis, using the DHBV genome. DHBV DNA can initiate infection of either intact animals (when delivered by intrahepatic inoculation[18-20]) or of cultured human hepatoma cell lines[21,22] (following transfection with cloned viral DNA[23,24]). For the assay in animals, one-day-old ducklings were intrahepatically injected with mutant DNA, and 18-21 days later (to allow for horizontal spread of progeny virus) viral replication was scored by Southern blot analysis of liver DNA. In the cell culture assay, 3-5 days post-transfection, viral core particles were purified by density centrifugation or immunoprecipitation[25], and DNA extracted from the cores was analysed by blot hybridization. Authentic DHBV replication generates a characteristic array of DNA forms, including open circular and linear duplexes and a heterogeneous array of linear single-stranded and partially duplex circular forms (see, for examples, Fig. 2*b*, lanes 2 and 4). Site-specific mutations were generated using synthetic oligonucleotides[26]. Whenever possible, changes in *pol* were made so as not to affect *core*.

In this strain of DHBV, the *core-pol* overlap is 305 nucleotides (nt) with two AUG codons in the *pol* frame; the A residue of the first AUG is designated position 1 (Fig. 2*a*). Note that the *pol* frame is open for 60 nt 5′ to its first AUG. We first constructed a frameshift mutant in a region near the 3′ end of the *core-pol* overlap (mutant 253, Fig. 2*a*) by deletion of a single nucleotide at that position, which places a termination codon in the *pol* frame 10 nt downstream of the mutation. None of the four ducks inoculated with 253 DNA became infected, and no viral DNA synthesis was observed after introduction of 253 DNA into cultured cells (Fig. 2*c*). This indicates that sequences upstream of this site are required for production of *pol* gene products.

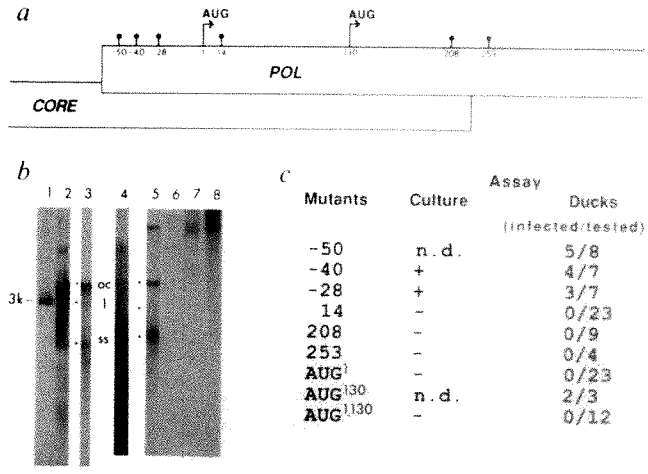


Fig. 2 Effects of mutations in the *pol* frame. *a*, Schematic representation of the *core* and *pol* overlap. Open boxes represents ORFs. In the overlap, the two AUG codons found in *pol* are marked by arrows. Individual nonsense mutations were made at the positions indicated by the closed circles (except mutant 253 which is a frameshift mutant). Each mutant contains sequence changes in both *core* and *pol* as follows: mutant 253, GCT (Ala) to GC in *pol*; mutant 208, CGA (Arg) to TGA (opal) in *pol* and TCG (Ser) to TTG (Leu) in *core*; mutant 14, TTG (Leu) to TAG (amber) in *pol* and ATT (Ile) to ATA (Ile) in *core*; mutant −28, TATca (Tyr) to TAAgc (ochre) in *pol*, and TCA (Ser) to AGC (Ser) in *core*; mutant −30, TGCgt (Cys) to TGAga (opal) in *pol* and CGT (Arg) to AGA (Arg) in *core*; mutant −40, TTTct (Phe) to TAAgt (ochre) in *pol* and TCT (Ser) to AGT (Ser) in *core*; mutant AUG1, AUG (Met) to ACG (Thr) in *pol* and GAT (Asp) to GAC (Asp) in *core*; mutant AUG130, AUG to ACG in *pol* and TAT (Tyr) to TAC (Tyr) in *core*. *b*, Southern blot of the replicative forms of viral DNA synthesized in duck livers or tissue culture following transfection. A DHBV probe was used to detect viral-specific sequences. Liver DNA samples from ducks tested with the wild-type DHBV DNA (lane 2) and the mutant −28 DNA (lane 3), and mutant −28 DNA from cytoplasmic cores extracted from transfected Huh7 cells (lane 4) are shown. DNA from cores prepared from HepG2 cells transfected with DNA of wild-type (lane 5), mutant 14 (lane 6), AUG1 mutant (lane 7) and AUG1,130 double mutant (lane 8) are also shown. Three major replicative forms of viral DNA are indicated by black dots; 'oc', open circular; 'l', linear; 'ss', single stranded. A linear 3 kbp DHBV DNA marker is shown in lane 1. *c*, Summary of DHBV mutants assayed both in tissue culture and in ducks. The results of duck inoculation experiments are shown to the right. '+' and '−', Positive and negative for pol function in HepG2 and/or Huh7 cells respectively. n.d., Not determined. The AUG1 and AUG1,130 mutants and mutant 14 all expressed a low level of pol activity detectable only after a long exposure of the autoradiograms; however, this activity does not support detectable viral replication in ducks.

To define further the boundaries of sequences required for *pol* expression, we analysed the behaviour of mutants with lesions in the *core-pol* overlap. To explore the 5′ boundary, several nonsense mutations were introduced in the *pol* ORF 5′ to its first AUG. Three of these, at positions −50, −40 and −28 relative to this AUG, do not affect the sequence of the *core* product, and all are viable in ducks and cultured cells (Fig. 2). Representative data for mutant −28 are shown in Fig. 2*b*, lanes 3 and 4. These data indicate that translation of pol must begin downstream of position −28. To map the 3′ boundary, nonsense mutations at positions 14 and 208 of pol were similarly analysed. As summarized in Fig. 2*c*, neither mutant grew in ducks and neither produced replicative intermediates in culture (see Fig. 2*b*, lane 6 for mutant 14). Therefore, sequences critical for *pol* expression are localized within a 42-nt region from −28 to +14 in the *core-pol* overlap. As this region contains an AUG codon, the contribution of this AUG to the generation of polymerase activity was addressed by further mutational studies.

If an internal initiation model is correct, the above results indicate that expression of the *pol* gene probably depends on the first of the two AUGs. When we changed the second AUG (AUG 130) to and ACG codon (without affecting *core*), two of three ducks tested positive for viral growth (Fig. 2*c*, mutant AUG^{130}), indicating that the second AUG is dispensable for *pol* function. However, when the first AUG of the *pol* ORF was mutated to an ACG codon (again without affecting *core*), the mutant did not replicate either in culture or in animals (Fig. 2*b*, lane 7, and Fig. 2*c*). Furthermore, a DHBV mutant with both of the AUG codons changed to ACG also failed to replicate in ducks (Fig. 2*c*, mutant $AUG^{1,130}$) or in culture (Fig. 2*b*, lane 8). These data suggest that the first AUG codon in the 42 bp region defined above is critical for viral *pol* expression or function. These results do not, however, exclude the possibility of ribosomal frameshifting within a small window from −28 to the first AUG, if this methionine was critical for *pol* function.

To define further the sequences in the *core-pol* overlap required for synthesis of pol protein, we constructed two amber mutants in the *pol* ORF at position −9 and −3 relative to the first AUG codon. When assayed, neither mutant replicated in ducks or in tissue culture (Fig. 3*a*). But as both contain a missense change in the *core* gene as well, the effects of the amber mutations on *pol* expression are ambiguous. A complementation assay was therefore developed to test the polymerase function independently of core function in tissue culture. A DHBV clone, DpA1, was constructed with multiple *pol* mutations, including the two AUG changes (at positions 1 and 130) described above and a 395-bp insertion downstream, yet maintaining the *core* amino-acid sequences intact (see Fig. 3*b*). This mutant expressed normal levels of viral mRNAs and core proteins (data not shown). As expected, the mutant is pol^-: when transfected alone into cells, DpA1 DNA produced no viral replicative intermediates (Fig. 3*c*, lane 2). The same result was observed for the −3 amber *pol* mutant (Fig. 3*c*, lane 3), but when this mutant DNA was cotransfected with DpA1, replicative forms of viral DNA were detected (Fig. 3*c*, lane 4). Similar results were obtained with the −9 amber mutant (data not shown).

It is very unlikely that DNA recombination can cause this result. No replicative forms were seen in control cotransfection experiments using two *pol* mutants, that is, DpA1 plus *pol*- mutant 253 or 208 (data not shown). In addition, to generate wild-type DNA from the −3 *pol* mutant and DpA1, recombination must occur within 2 bp (see Fig. 3*b*). To exclude DNA-DNA recombination further, however, we conducted an RNA-DNA cotransfection experiment. RNA containing only *core* sequences was synthesized *in vitro* using SP6 RNA polymerase and introduced into cells together with DNA carrying either the −9 or the −3 *pol* lesions. The products of viral polymerase activity were present in cell extracts collected 48 h post-transfection (Fig. 3*d*).

Finally, we investigated whether *pol* production is dependent upon translation of the antecedent *core* gene, a strong prediction of the frameshifting model. We constructed the plasmid Dfs1, which has a frameshift mutation in the *core* ORF, terminating *core* translation well upstream of the *core-pol* overrlap region (Fig. 4*a*). As expected, this mutant is not replication-competent in culture (Fig. 4*b*, left panel, lane 2). The ability of this mutant to generate a functional *pol* product was then tested using complementation assays. When this clone was cotransfected with DNA of the known pol^- mutant DpA1 (Fig. 3*b*), viral replication intermediates were produced (Fig. 4*b*, left panel, lane 1), indicating that Dfs1 does indeed produce pol proteins.

The possibility of DNA-DNA recombination was excluded by showing that the defect in Dfs1 could be complemented by the provision of the core function alone in an RNA-DNA cotransfection experiment. As shown in Fig. 4*b* (right panel, lane 1), when Dfs1 DNA was cotransfected with *core* mRNA, viral replicative intermediates were produced. As a control, cotransfection of Dfs1 with the SP6-core DNA template (which does not contain the DHBV promoter), did not allow the production of pol (the right panel, lane 2); thus any homologous recombination between incoming DNA molecules is not sufficient to allow detectable marker-rescue of the *core* frameshift mutation. These results indicate that *pol* expression does not depend on translation of *core*.

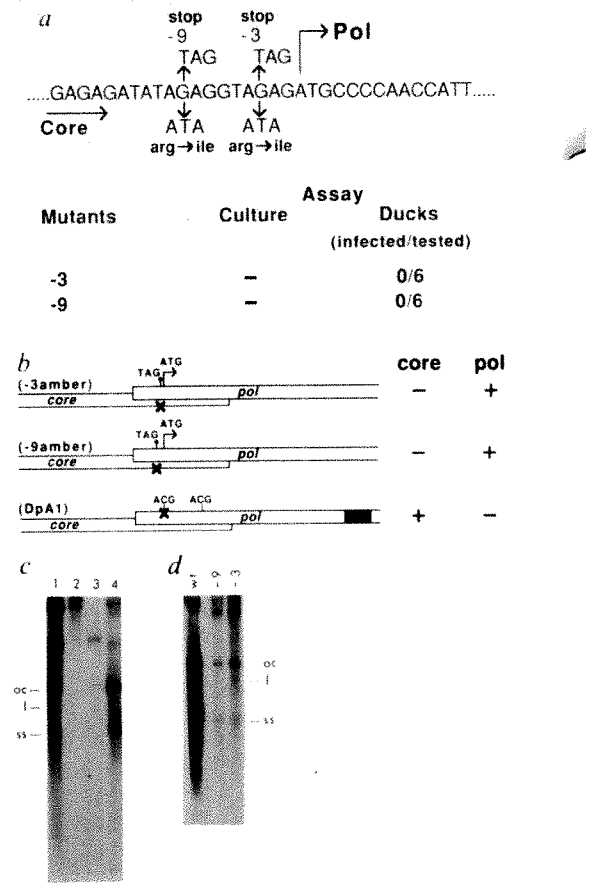


Fig. 3 Assay for *pol* function of two *pol* amber mutants immediately 5' to the first *pol* AUG. *a*, Assays in animals and cell culture. Point mutations were created individually at positions −3 and −9 in the *core-pol* overlap. As shown in the sequence both mutants create simultaneously a stop codon in the *pol* frame (arrow directed up) and a missense mutation in the *core* frame (arrow down). *b*, Schematic structure of the −3 and −9 *pol* amber mutants and the DpA1 clone. Both amber mutants bear a missense lesion in *core* (leading to a nonfunctional core protein) indicated by the '×'. The DpA1 bears two lesions in *pol* that affect the phenotype, one at the first AUG codon (the '×') and the other an insertion in the middle of *pol* (filled box). The second AUG to ACG mutation at +130 does not affect viral function (Fig. 2). The phenotypes determined by tissue culture assay are shown to the right. *c*, Complementation assay by DNA-DNA cotransfection. Lane 1, wild-type DNA alone; lane 2, DpA1 DNA alone; lane 3, the −3 amber mutant DNA alone; lane 4, DpA1 plus −3 amber mutant DNA. *d*, Complementation assay by RNA-DNA cotransfection. 'wt', Wild-type DNA alone; '−9', SP6 core RNA and −9 amber mutant DNA; '−3', SP6 core RNA and −3 amber mutant DNA.
Methods. For the construction of DpA1, a 395 bp fragment from *Staphylococcus aureus* protein A gene was cloned into the *pol* ORF at the *Kpn*I site (near the *S* gene AUG) using the $AUG^{1,130}$ mutant clone. DNA-DNA cotransfection was performed by the use of same amount of both DNAs (15 μg each in a 100-mm dish). The RNA-DNA cotransfection used poly-L-ornithine[37] at 20 μg ml^{-1} and only HepG2 cells were used for this assay.

We have presented several lines of evidence to indicate that the hepadnaviral reverse transcriptases are not made as core-pol fusion proteins by ribosomal frameshifting. Hepatitis B viruses thus differ from all well-studied retroviruses and retrotran

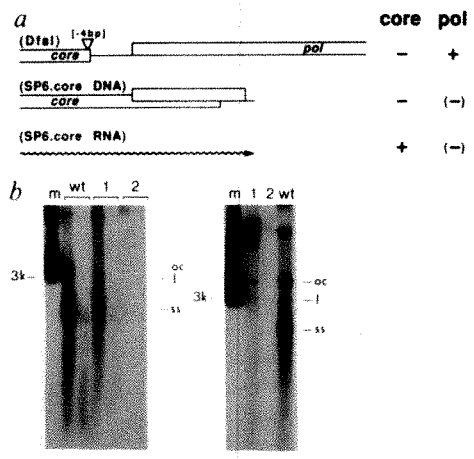


Fig. 4 Complementation assay with the *core* frameshift mutant Dfs1. *a*, Schematic structure of the Dfs1, SP6 core DNA and SP6 core RNA. In Dfs1, fill-in of the unique *Nsi*I site in *core* causes frameshift and premature termination (two codons downstream of the frameshift site) in the *core* frame. The SP6 core plasmid DNA was constructed in pSP65; it contains only part of the *pol* gene and has no eukaryotic promoter. The phenotypes of each construct as assayed in tissue culture are shown to the right. *b*, Complementation assay by DNA-DNA and RNA-DNA cotransfection. Cytoplasmic cores were prepared from transfected HepG2 cells by immunoprecipitation. In the left panel, lanes are shown in pairs; for each pair (wt, 1, and 2) the left lane shows DNA prepared from immunoprecipitated cores. The right lane shows the same core preparations extracted with phenol before proteolysis; loss of DNA from the aqueous phase indicates its association with a 5′ terminal protein[35]. Lanes 1, Dfs1 plus DpA1 DNA; lanes 2, Dfs1 DNA alone. In the right panel: lane 1, SP6 core RNA plus Dfs1 DNA; lane 2, SP6 core DNA plus Dfs1 DNA. m, The DHBV 3 kbp linear marker; wt, wild-type.

Methods. For the construction of Dfs1, the unique restriction site *Nsi*I (67 codons into the *core* gene) in the *core* gene of the genomic DHBV clone was filled in by bacteriophage T4 polymerase and recircularized with T4 ligase. For the construction of SP6 core clone, a DNA fragment containing the complete *core* gene from the *Acc*I site (in the pre-*C* region) to *Ava*I site (downstream of the *C* gene) was cloned into pSP65 under contaol of the SP6 promoter.

posons in that the reverse transcriptase function is not generated as a polyprotein with upstream nucleocapsid domains. A similar mechanism of pol generation has, however, been suggested for the cauliflower mosaic virus of plants[27], in which the *pol* gene can be separated from the upstream cistron without deleterious effects.

In all elements yet encountered that carry out reverse transcription, the reaction is confined to a particulate nucleoprotein structure that presumably serves to prevent the enzyme from gaining access to cellular mRNAs. In retroviruses, frameshifting also solves the problem of selective inclusion of the pol protein into viral particles, as the *gag* domain of the gag-pol fusion protein can interact with other gag proteins during assembly[28]. In the light of our results, it is clear that hepadnaviruses must solve this problem in some other way, for instance by protein-protein interactions between core and pol, or by direct interactions between the pol protein and pregenomic RNA.

Our data (and those of H. Schlicht, G. Radziwill and H. Schaller, personal communication) indicate that the HBV polymerase is synthesized by initiation at the first *pol* AUG, a view which is further corroborated by the conservation of this AUG in all members of the hepadnavirus family. Such initiations would probably occur from the internal position of the *pol* AUG in pregenomic RNA. Several models for this initiation can be envisioned: the most orthodox interpretation is as an extreme example of 'leaky' scanning[29], in which only the rare ribosomes that bypass the upstream AUGs during scanning from the 5′ cap become available to the *pol* AUG. The *pol* initiator, however, is itself in a suboptimal sequence context for initiation, with a C residue rather than a purine in the +4 position (see Fig. 3*a*). Also, in the 660 nt of the pregenomic RNA 5′ to this initiator, there are 15 other AUGs in all three reading frames (not including the *pre-core* AUG). These considerations argue against a simple extension of the leaky scanning model. In addition, experiments with the *core* frameshift mutant Dfs1 also rule out the possibility of ribosomes 'reaching back' for the *pol* AUG after terminating at the *core* terminator. Such 'backwards scanning' has been demonstrated in certain other bicistronic mRNAs with overlapping genes[30], but in Dfs1 *core* translation terminates 112 codons upstream of the *pol* AUG, and yet expression of *pol* is unaffected (Fig. 4).

We favour a model of *de novo* initiation at the *pol* AUG. One possibility is that the region flanking the *pol* initiator may have features that facilitate the direct entry of ribosomes or binding of translation initiation factors. Such sequences have been proposed for the cap-independent translation of poliovirus mRNA in which translation initiation occurs at an AUG downstream of an approximately 750-nt region containing seven other AUGs[31]. The use of the *pol* AUG, by whatever mechanism, seems to be low efficiency, as only minute quantities of polymerase protein have been detected in virions by direct biochemical and immunologic tests[16,17]. This may be of importance in viral gene regulation, as in retroviruses mutations which lead to overexpression of the *pol* precursor are highly deleterious to viral growth[32].

A growing number of examples exists of cellular messages which may use internal initiation. A recently described transcript of the human *c-abl* oncogene[33], for example, has a 5′ noncoding region of over 1 kb, with 13 AUGs; a similar situation exists upstream of an *Antennapedia* transcript in *Drosophila*[34]. It is reasonable to assume that these unusual coding organizations have potential regulatory significance. The further study of HBV *pol* and other eukaryotic genes displaying internal initiation should allow a better mechanistic understanding of this intriguing process.

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Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells

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The CD4 antigen has been subverted as a receptor by the human and simian immunodeficiency viruses (HIV-1, HIV-2 and SIV)[1–4]. Several groups[5–9] have reported that recombinant, soluble forms of the CD4 molecule (sCD4) block the infection of T lymphocytes by HIV-1, as CD4 binds the HIV envelope glycoprotein, gp120, with high affinity[10,11]. We now report that sCD4 blocks diverse strains of HIV-1, HIV-2 and SIV, but is less effective for HIV-2. The blocking effect is apparent even after adsorption of virions to CD4 cells. Soluble CD4 prevents HIV infection of T-lymphocytic and myelomonocytic cell lines, but neither sCD4 nor anti-CD4 antibodies inhibit infection of glioma and rhabdomyosarcoma cell lines.

We first investigated the capacity of sCD4 to block infection of diverse strains of HIV-1, HIV-2 and SIV. Table 1 shows that sCD4 preparations blocked the infectivity of each strain tested, but the HIV-2 isolates required 25-fold higher concentrations of sCD4 than HIV-1 to achieve comparable virus neutralization. In a separate experiment, virus strains were treated with sCD4 and titrated for surviving plaque-forming units (p.f.u.) on MT-4 cells[12] with the same results. Table 1 also shows that syncytium formation between HIV-producing and uninfected cells[1] was also inhibited, but was less sensitive to sCD4 than the neutralization of HIV or SIV virions was. This difference may result from the higher viral antigen 'load' present in the cell fusion reaction, which is also less sensitive to most neutralizing antibodies[13]. The relative sensitivity to sCD4 inhibition of the different virus strains was similar for syncytium formation and HIV titration.

The difference in sensitivity to sCD4 between HIV-1 and HIV-2 may reflect a high concentration of free gp120 in HIV-2 stocks, a higher density of gp120 on HIV-2 virions, or a higher binding affinity between HIV-1 gp120 and CD4. We used vesicular stomatitis virus (VSV) pseudotypes bearing the envelope glycoproteins of HIV to investigate whether free gp120 competed with sCD4 neutralization of virions. VSV(HIV) pseudotypes depend on CD4 surface receptors for binding to and entry into host cells[1]. Once inside the cell, however, the pseudotype replicates as VSV, providing a simple plaque assay for viral antigen-cell receptor interactions.

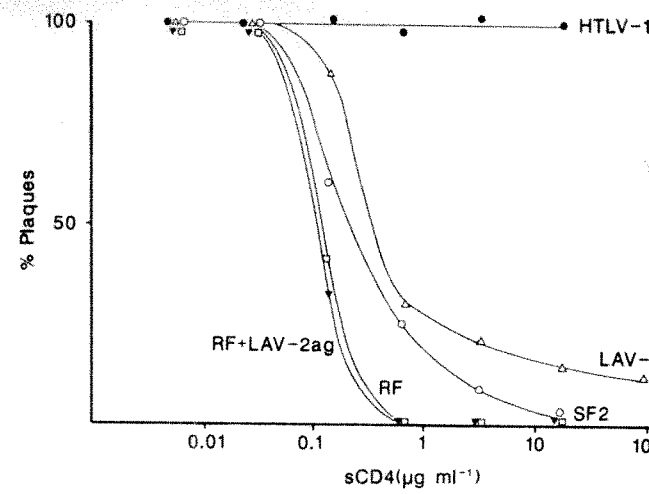


Fig. 1 Inhibition of VSV(HIV) plaque formation by sCD4. VSV pseudotypes bearing the envelope glycoproteins of HIV and HTLV-1 were prepared as described[1,19,35]. Pseudotype stocks were diluted to 10^3 p.f.u. ml^{-1} and were incubated for 1 h at 37 °C with sCD4 at the concentrations indicated. The reduction in plaque count was scored after assay on adherent CEM cells[1]. HTLV-1 was the PL strain[35], LAV-2_{ROD} represented HIV-2, HTLV-IIIRF (RF) and SF2 (ARV-2) represented HIV-1 strains. Undiluted LAV-2_{ROD} supernatant medium (LAV-2 ag) was added to VSV(RF) to test the effect of excess HIV-2 antigen on plaque inhibition by sCD4.

Figure 1 shows that sCD4 was effective in neutralizing both HIV-1 and HIV-2 pseudotypes. The pseudotype of the HIV-1 strain (HTLV-IIIRF) however was more sensitive to sCD4 than the HIV-2 strain tested (LAV-2), and a second HIV-1 strain (SF2, formerly known as ARV-2) was intermediate. SF-2 represents an isolate that is unusually sensitive to antibody neutralization by human sera[13], and it may react with sCD4 differently from other HIV-1 strains. The 80% blocking titres of sCD4 for VSV(HIV) pseudotypes were similar to those for HIV infectivity, being approximately 0.3 μg ml^{-1} for VSV(HTLV-IIIRF) and 5 μg ml^{-1} for VSV(LAV-2). As a control for the specificity of sCD4 neutralization, VSV(HTLV-1) pseudotypes were similarly reacted with sCD4 and were not inhibited at any concentration.

Evidence that the observed difference in sCD4 sensitivity between HIV-1 and HIV-2 is not related to soluble gp120 was obtained from a competition experiment in which supernatant medium from an LAV-2 releasing culture was added to VSV(HIV-1) before sCD4 treatment. The HIV-2 containing medium should have no effect on the VSV(HIV) plaque count, but should compete with VSV(HIV) particles for binding sCD4. LAV-2 supernatants were added to VSV(HTLV-IIIRF) at a concentration that required at least 40 μg ml^{-1} sCD4 for its infectivity to be inhibited. Despite this 'overload' of HIV-2 antigen, there was no reduction in sensitivity of VSV(HTLV-IIIRF) to sCD4 inhibition (Fig. 1). Addition of HIV-1 supernatant had no effect on VSV(HIV) inhibition either (data not shown).

We next investigated whether sCD4 would inhibit HIV after virions had bound to cellular CD4 receptors. Serial dilutions of HIV-1 were treated with sCD4 either before adsorption or one hour after adsorption to T cells. The results (Table 2) demonstrate the potential of sCD4 to block HIV post-adsorption, albeit some 50-fold less efficiently than if added to HIV before adsorption. The blocking effect indicates that even after binding to

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Table 1 Soluble CD4 inhibition of infection and syncytium formation by HIV-1, HIV-2 and SIV strains

Virus		Inhibition titre of sCD4 ($\mu g\ ml^{-1}$)		
		Infection TCID	Plaque assay	Syncytium formation
HIV-1	HTLV-IIIB	0.3	0.3	1.5
	HTLV-IIIRF	0.3	0.3	1.5
	SF33	0.12	0.12	0.6
	CBL4	0.3	ND	1.5
HIV-2	LAV-2_{ROD}	7.6	7.6	38
	CBL20	7.6	7.6	38
SIV	SMM (mangabey)	0.6	ND	7.6
	MAC (macaque)	0.6	ND	7.6
	AGM (green monkey)	0.6	ND	7.6

Soluble CD4 representing the whole of the extracellular domain was prepared as previously described[8]. For infection inhibition (neutralization) assays, serial dilutions of sCD4 were incubated at 37 °C for one hour with 1,000 T-cell infectious doses (TCID) of HIV or SIV. The surviving infectious virus was titrated by plating dilutions of the sCD4-treated virus on C8166 T cells and recording virus replication by the appearance of syncytia and reverse transcriptase activity 48–72 hours later[19]. Virus treated with sCD4 was also titrated in the MT4 cell HIV plaque assay[12]. For syncytium formation, chronically infected H9 cells were mixed with uninfected C8166 indicator cells and multinucleated syncytia were counted after incubation overnight[19]. The concentration of sCD4 neutralizing over 80% of TCID and p.f.u., and causing 80% inhibition of syncytium formation are tabulated for each virus strain. ND, not determined.

Table 2 Reduction of HIV-1 infection by sCD4 after adsorption of virus to T-lymphocytes scored by syncytial focus assay

Virus dilution	No. sCD4	sCD4 added before HIV adsorption	sCD4 added after HIV adsorption
10^{0}	con, con	1, 2	105, 85
10^{-1}	203, 240	0, 0	10, 13
10^{-2}	25, 41	0, 0	3, 1
10^{-3}	4, 2	0, 0	0, 0

HIV-1 (HTLV-IIIRF) was titrated by a syncytial focus assay as follows: C8166 cells (10^6 cells in 0.5 ml RPMI/1640 medium with 10% fetal calf serum) were plated into 12-mm tissue culture wells in 24-well plate wells (Nunclon, Denmark) coated with 50 $\mu g\ ml^{-1}$ poly-L-lysine to form a monolayer. After 20 min at room temperature, appropriate virus dilutions were added and adsorbed for 1 h on ice. The monolayers were washed in culture medium to remove unabsorbed virus and were incubated in medium for 1 h, washed again, and overlaid with 1 ml culture medium containing 0.9% molten Sea Plaque agarose (FMC Bioproducts). After 42 h incubation at 37 °C, 1 ml agarose containing 0.0032% Neutral Red was added and at 48 h discrete focal areas of syncytia were counted by low power magnification (×20). The linear dose-response of syncytial foci in the absence of sCD4 indicated that each focus resulted from a single infectious unit of HIV and the focus titre correlated well with HIV titration by end-point dilution on C8166 cells in suspension ($TCID_{50}$). To measure the inhibitory effect of sCD4, 40 $\mu g\ ml^{-1}$ protein was added to serially diluted HIV for 1 h on ice in culture medium before adsorption, or was added to the washed cell monolayer on ice following virus adsorption. The numbers represent focus counts of duplicate wells for each sCD4 treatment and virus dilution. Con, confluent syncytia.

cellular CD4, HIV infection can be inhibited by the competing soluble homologue, before internalization by a *p*H-independent fusion process[14–16].

HIV infection of T-lymphocytic[1,2,17] and monocytic[18,19] cells can be blocked by preincubating the cells with certain anti-CD4 monoclonal and polyclonal antibodies. Other cell types, notably glioma cell lines[20–22] and RD cells[23], can also be infected with high titres of HIV-1. HIV-susceptible glial cell lines, such as U138, are negative for CD4 surface expression by immunofluorescence, but express low levels of CD4 mRNA. (J.N.W., unpublished observations). Preliminary experiments indicated that anti-CD4 antibodies did not inhibit HIV infection. The low density of CD4 expression on glial cells might prevent anti-CD4 attaching with sufficient avidity to compete with HIV binding. By using sCD4 as an inhibitory reagent binding to the virion glycoproteins, gp120, rather than to the cellular receptor molecule, the question of whether CD4 is required for infection of these cells can be addressed more clearly.

Table 3 shows that U138 astroglioma cells, but not U87 astroglioma cells, can be infected with the HTLV-IIIRF strain of HIV-1. The TE671 cell line can also be infected with HIV[20]. This line was thought to be derived from a human medulloblastoma[24], but is now known to be the RD rhabdomyosarcoma cell line[25]. A relatively high input of virus required for U138 and RD cell infection ($>10^3$ T-lymphocyte infectious units), and the infection is only weakly productive. Not enough virions are released into the supernatant medium of infected U138 or RD cultures to register as positive in reverse transcriptase or p24 antigen assays, and it seems that only a small proportion of cells contain HIV antigens or proviral DNA[20–22]. Virus can readily be rescued by cocultivation with a fully permissive T-cell line however, such as C8166 or by transfer of the supernatant medium of infected glioma cell cultures to C8166 cells. Incubation of HIV-1 with sCD4 before plating failed to block infection of brain or muscle cell lines at 18 $\mu g\ ml^{-1}$ or even 180 $\mu g\ ml^{-1}$. In a parallel experiment, monoclonal antibodies reacting to CD4 antigen (Leu3a, F101-5) efficiently blocked HIV infection of $CD4^+$ lymphocytic and monocytic cells but also failed to block infection of brain tumour cells. In contrast, a human serum containing antibodies to gp120, gp41 and gag antigens neutralized HIV for all cell types (Table 3). It is most unlikely that input virus has been carried over in the brain cell cultures, in view of the serial passage and trypsinization of these cells, and that the resistant U87 glial cell line did not yield rescued virus upon subsequent cocultivation with T cells (Table 3). Moreover, proviral DNA has been detected after HIV infection of these cell types[20,21]. We therefore conclude that HIV infects glial and muscle cells in a way that is not mediated by CD4.

Soluble CD4 is under investigation as a therapeutic agent in HIV-infected subjects. One concern is that sCD4 might itself be immunosuppressive, reacting *in vivo* with the apparent natural ligand for CD4, the major histocompatibility complex (MHC) class II antigens[4,26]. Little or no effect of sCD4 on class II-restricted T-cell interactions is seen *in vitro* however[7]. The primary recognition site on CD4 for gp120 is close to the Leu3a antigenic epitope[4,27] and has been mapped to the N-terminal immunoglobulin V-like domain[28,29]. An sCD4 fragment or peptide containing this site but which was not involved in immune recognition would be desirable, though the evolutionary conservation among primate species of the gp120/Leu3a site suggests that this epitope may be important for normal functions of the CD4 molecule[30].

Our results show that neither sCD4 nor Leu3a antibody block infection of certain brain and muscle cell lines. These unexpected results indicate that HIV may not require cell surface CD4, nor perhaps viral gp120, to gain entry to these cells *in vitro*. It would seem unlikely to depend on an artefactual 'transfection' as described for bacteriophage[31] as DEAE-dextran was not used to enhance virion uptake, and the RD/TE671 cultures became infected with an input as low as 0.05 T-cell infectious doses per cell (Table 3). HIV and VSV(HIV) pseudotypes are unable to infect mouse cells expressing human CD4 or to induce cell fusion although the virions bind to the CD4 molecules[32]. A second membrane molecule may therefore be required for fusion and viral penetration. In view of the relatively low efficiency of infection of glioma and myoblast cells compared to $CD4^+$ T

Table 3 Effect of sCD4 and anti-CD4 antibody on HIV-1 infection of different human cell types

Cell type	Input HIV (TCID)	Human anti-HIV-1 SF	Human anti-HIV-1 RF	Leu3a anti-CD4 SF	Leu3a anti-CD4 RT	sCD4 180 μg ml^{-1} SF	sCD4 180 μg ml^{-1} RT	Control SF	Control RT
T cell									
C8166	5×10^3	−	−	−	−	−	−	+ + +	20,000
Myelomonocyte									
U937	5×10^5	ND	ND	−	−	−	−	+ + +	27,000
	5×10^3	−	−	−	−	−	−	+ + +	55,000
HL60	5×10^5	ND	ND	−	−	−	−	+ +	49,000
	5×10^3	−	−	−	−	−	−	+ +	9,000
Glioma									
U138	5×10^5	−	−	+ +	21,000	+ +	23,000	+ +	23,000
U87	5×10^5	−	−	−	−	−	−	−	−
Muscle									
RD/TE671	5×10^5	−	−	+ +	18,000	+ +	26,000	+ +	19,000
	5×10^3	−	−	+ +	9,000	+ +	20,000	+ +	18,000

HIV-1 (HTLV-IIIRF strain) was incubated with anti-HIV-1 antibody or with sCD4 for 1 h at 37 °C before plating on 10^6 cells of the types indicated. Syncytium formation (SF) and reverse transcriptase (RT) activity in the medium was scored 5 days post-infection for the T-cell and myelomonocyte cultures. For the glial and myoblast cultures there were no detectable SF or RT activity as previously described[20–23]. After seven days and two trypsin-mediated passages of the glial and myoblast cells, C8166 cells were added to the cultures, and SF and RT activity was scored 3 days later. Virus production for cells exposed to HIV-1 (not treated with anti-CD4 or sCD4) was also detected after 3 weeks and 6 passages in U138 and RD/TE671 cells, but not U87 cells. The glial cells expressed glial fibrillar acidic protein, confirming their provenance. SF scores: +++, >50% giant syncytial cells in culture; ++, 10–50% syncytial cells; +, 1–9% syncytial cells; −, no discernable syncytia. RT scores in c.p.m. acid precipitable [^{3}H]thymidine incorporation; −, <1,000 c.p.m. ND, not determined.

lymphocytes, HIV infection might occur by gp41-mediated membrane fusion in the absence of specific gp120-CD4 receptor interaction.

It is not clear whether HIV infection of astroglial cells has a role in HIV encephalopathy (as infection is mainly detectable in microglial and endothelial cells[33,34]) or whether muscle infection is related to the weight loss and wasting seen in AIDS. Although our results may be restricted to cells *in vitro*, HIV infection of glial and muscle in the presence of excess sCD4 merits further investigation, given the interest in developing sCD4 as a therapeutic agent.

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Productive dual infection of human CD4$^+$ T lymphocytes by HIV-1 and HHV-6

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Although infection by HIV-1 (refs 1 and 2) has been implicated as the primary cause of AIDS and related disorders[3,4], cofactorial mechanisms may be involved in the pathogenesis of the disease. For example, several viruses commonly detected in AIDS patients and capable of transactivating the long terminal repeat of HIV-1, such as herpesviruses[5–7], papovaviruses[7], adenoviruses[8] and HTLV-I (ref. 9), have been suggested as potential cofactors. Another candidate is human herpesvirus-6 (HHV-6, originally designated human B-lymphotropic virus)[10–12], which has not only been identified in most patients with AIDS by virus isolation[10,13,14], DNA amplification techniques[15] and serological analysis[16], but is also predominantly tropic and cytopathic *in vitro* for CD4$^+$ T lymphocytes[17,18]. Here we demonstrate that HHV-6 and HIV-1 can productively co-infect individual human CD4$^+$ T lymphocytes, resulting in accelerated HIV-1 expression and cellular death. We also present evidence that HHV-6 transactivates the HIV-1 long terminal repeat (LTR). These observations indicate that HHV-6 might contribute directly or indirectly to the depletion of CD4$^+$ T cells in AIDS.

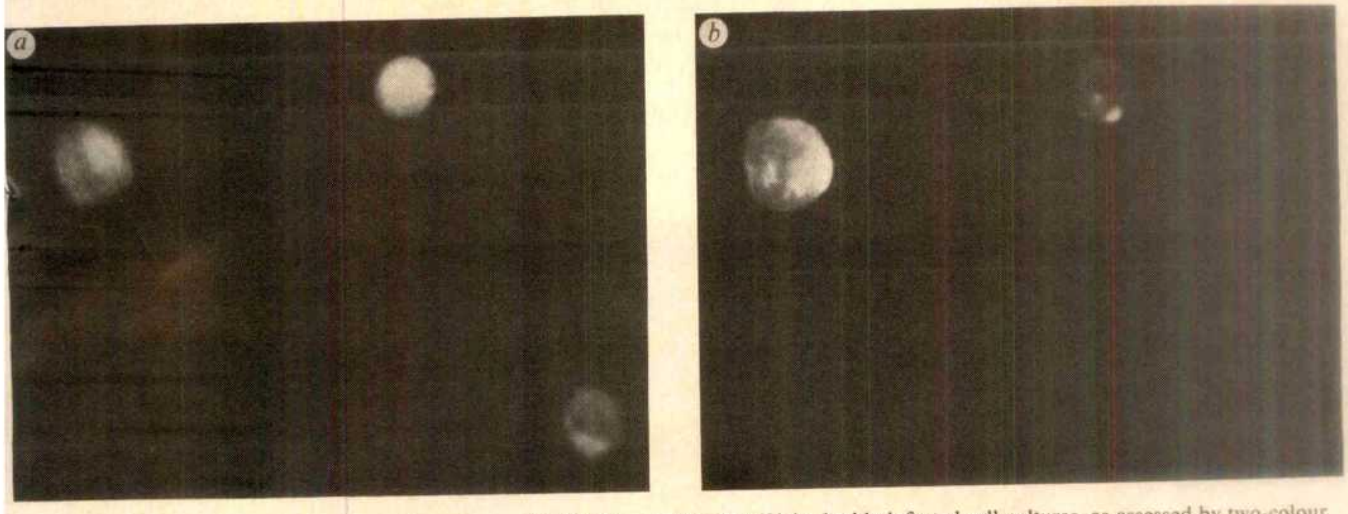


Fig. 1 Simultaneous expression of specific antigens of HHV-6 (*a*) and HIV-1 (*b*) in doubly infected cell cultures, as assessed by two-colour indirect immunofluorescence.

Methods. PBMC from healthy volunteers were isolated by Ficoll-Hypaque gradient centrifugation and cultured at 37 °C in a humidified atmosphere of 5% CO_2 at a density of 1×10^6 ml^{-1} in RPMI medium supplemented with 15% fetal bovine serum, 1 μg ml^{-1} phytohaemagglutinin-P and 5 μg ml^{-1} polybrene. Cells were washed with RPMI after 24 h, counted and exposed for one hour at 37 °C to HIV-1 alone (isolate HTLV-III$_B$), HHV-6 alone or to HIV-1 plus HHV-6; each virus was at an infectious multiplicity of ~0.3. Infected cells were then cultured at 10^6 per ml in RPMI supplemented with 10% fetal bovine serum and 20% of their original culture medium collected before infection. The absolute numbers of viable cells, CD4$^+$ cells and giant multinucleated cells (syncytia) were recorded at 2-day intervals. After removal of dead elements by Ficoll-Hypaque gradient centrifugation, viral antigen expression was evaluated in parallel by indirect immunofluoresence on cytocentrifuge preparations fixed for 5 min in cold acetone[18]. A murine monoclonal antibody against the gag p24 protein of HIV-1 (M26)[20] was used at 2 μg ml^{-1}; an anti-HHV-6 human serum (N828), extensively adsorbed with uninfected phytohaemagglutinin-activated PBMC, was used at a final dilution of 1:15. Phycoerythrin-conjugated (red) goat anti-mouse IgG and fluorescein-isothiocyanate-conjugated (green) goat-anti-human IgG polyclonal antisera (both specific for γ-chain; Kirkegaard and Perry Inc., Maryland), were used as second layer antibodies. Samples were visualized with a Leitz Ortholux II fluorescence microscope equipped with a ×40 dry objective. Two-colour immunofluorescence also demonstrated that cells infected with HHV-6 and HIV-1 displayed a CD4$^+$ T-cell phenotype.

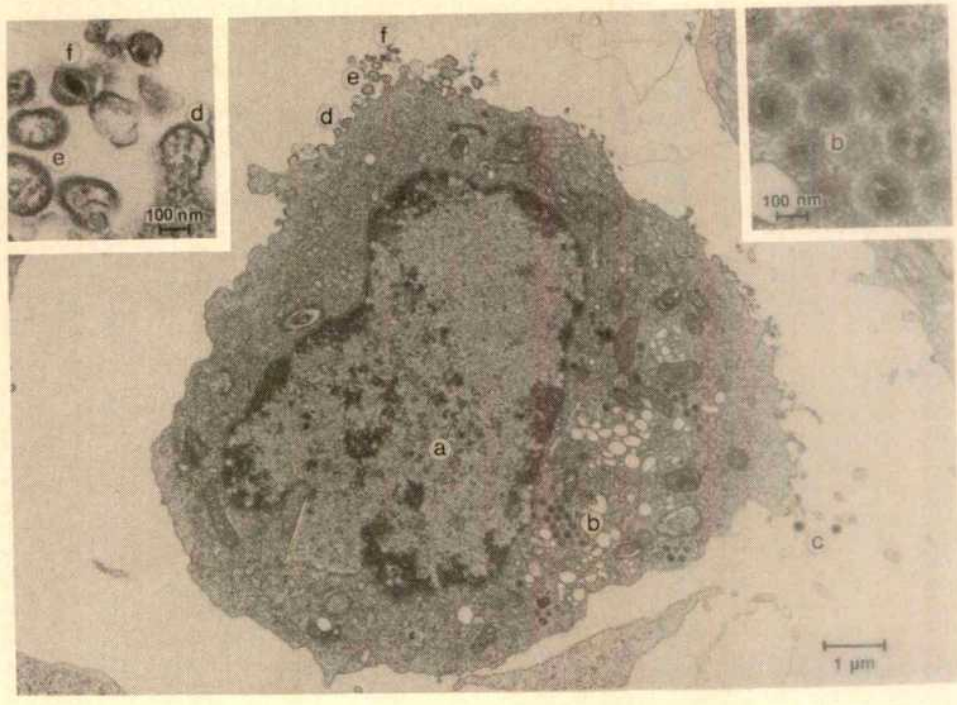


Fig. 2 Electron micrograph of a cell with the morphological features of an activated lymphocyte, eight days after simultaneous infection of PBMC cultures with HIV-1 and HHV-6. *a*, Unenveloped intranuclear HHV-6; *b*, unenveloped intracytoplasmic HHV-6; *c*, mature extracellular HHV-6; *d*, budding HIV-1 particles; *e*, immature extracellular HIV-1 (rarefied core); *f*, mature HIV-1 (dense core). Magnification is ×15,000 (detailed inserts, ×75,000). Cells were extensively washed with cold PBS, centrifuged at 1,500 r.p.m. and fixed with 1.25% glutaraldehyde, 0.1 M sodium cacodylate, 0.5×PBS for electron microscopy.

Freshly isolated normal human peripheral blood mononuclear cells (PBMC) were activated *in vitro* with purified phytohaemagglutinin and subsequently co-infected with HIV-1 and HHV-6. Two-colour indirect immunofluorescence was used to detect specific viral antigens and demonstrated that individual cells could serve as a simultaneous target for both viruses (Fig. 1). Electron microscopy illustrated the productive nature of this dual infection, showing the complete pathway of HHV-6 and HIV-1 maturation within individual lymphocytes. HHV-6 was visible as both immature (namely, nuclear or cytoplasmic unenveloped nucleocapsids) and mature extracellular particles (Fig. 2*a*–*c*). Budding, extracellular immature and mature HIV-1 virions could be recognized if associated with the same cell (Fig. 2*d*–*f*).

The expression of viral messenger RNA, as analysed by northern blotting, was qualitatively unaltered in dually infected cultures and the viruses released were found to be infectious for normal human PBMC (data not shown). As summarized in Table 1, however, HIV-1 antigen expression was consistently accelerated in the course of co-infection with HHV-6. It must

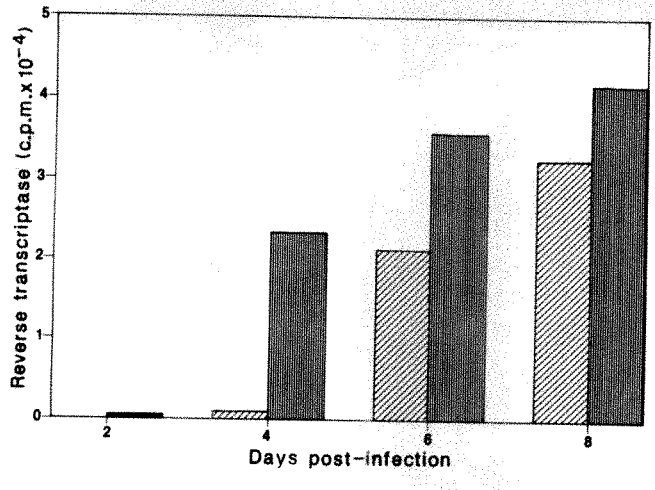


Fig. 3 Reverse transcriptase activity in cultures of PBMC infected with HIV-1 alone (cross-hatched) or with HIV-1 and HHV-6 together (shaded). Details of infection and culture procedures are given in the legend to Fig. 1. Polyethyleneglycol-precipitated cell culture supernatant fluids were assayed for reverse transcriptase activity as previously described[2].

be emphasized that the majority of HIV-1-positive cells were simultaneously expressing HHV-6 antigens. Moreover, these immunofluorescence determinations probably underestimated the actual magnitude of the infection, as dead (presumably infected) cells were removed before testing. An accelerated expression of HIV-1 in dually infected cultures was further indicated by the earlier detection of reverse transcriptase activity (Fig. 3).

Consistent with the acceleration of HIV-1 expression, we also observed a more rapid induction of cytopathicity in the course of dual infection. The loss of $CD4^+$ T lymphocytes was significantly accelerated in dually infected PBMC cultures (Fig. 4*a*) and mathematical analysis of the data suggested a synergistic cytocidal action of HIV-1 and HHV-6 (Fig. 4*b*). A parallel decline of total cell viability was also observed in these cultures. For example, eight days after infection, the absolute numbers of viable cells were $4.3 \pm 0.5 \times 10^5$ with HIV-1 and $2.7 \pm 0.5 \times 10^5$ with HHV-6, as compared to $0.6 \pm 0.2 \times 10^5$ with both viruses together ($P < 0.01$). These observations indicate that, despite the documented down-regulation of CD4 molecules by HIV-1 gp120/160 glycoproteins[19], the chief mechanism for $CD4^+$ cell depletion in our cultures was a virus-induced cytopathic effect. Moreover, HIV-1 induced syncytia were identified earlier in doubly infected cultures (Table 1). Dual infection of $CD4^+$ T cells was also observed using established human $CD4^+$ T cell lines (not shown).

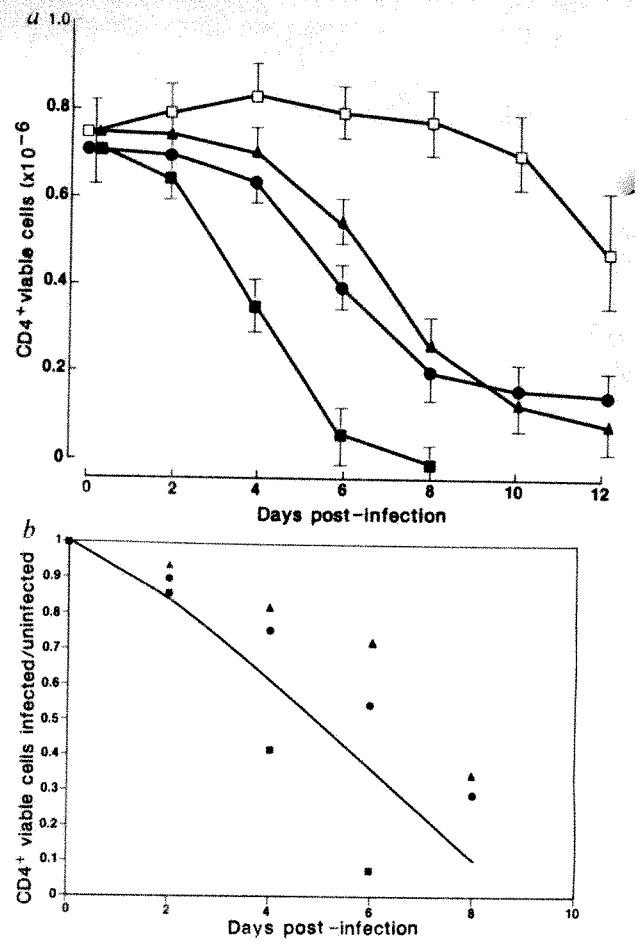


Fig. 4 Cytopathic effect in the course of co-infection of normal human PBMC by HIV-1 and HHV-6. Open square, uninfected; filled triangle, HIV-1 alone; filled circle, HHV-6 alone; filled square, HIV-1 plus HHV-6. *a*, Kinetics of $CD4^+$ cell viability in singly and doubly infected cultures. Data represent the averaged results (± standard deviation) from 3 separate experiments. *b*, Synergystic effect of HIV-1 and HHV-6 in inducing cytopathicity on normal PBMC cultures. All values are normalized with respect to uninfected control cultures (representing the ratio between viable $CD4^+$ cell counts in infected cultures and viable $CD4^+$ cell counts in uninfected cultures for each time point). The solid line represents the predicted curve in the course of an additive effect and was calculated according to the following formula: $N_t = p_t q_t$ (where p_t and q_t correspond to normalized $CD4^+$ cell counts at time t in cultures infected with HIV-1 and HHV-6 respectively). By day 4 or 6 post-infection, the observed mortality in dually infected cell cultures was 2–3 times greater than the values predicted for a simple additive effect.

Table 1 Viral antigen expression and syncytia formation in normal PBMC cultures infected with HIV-1, HHV-6 or both viruses simultaneously

	Virus used for infection*							
	HIV-1		HHV-6		HIV-1 plus HHV-6			
Day post-infection	HIV-1† %	S‡	HHV-6† %	S‡	HIV-1† %	HHV-6† %	HIV-1+HHV-6† %	S‡
2	—	–	—	–	0.7 (0.6)	—	—	–
4	1.7 (1.6)	–	6.7 (5.8)	–	10.7 (7.5)	14.7 (10.3)	9.3 (6.5)	–
6	9.3 (7.2)	–	30.7 (14.4)	–	38.3 (12.6)	45.7 (15.1)	24.0 (7.9)	+
8	22.7 (9.7)	+	68.3 (18.4)	–	61.0 (3.7)	82.7 (5.0)	50.3 (3.0)	+
10	30.3 (11.2)	+	80.0 (18.4)	–	NA‖	NA	NA	+

* Phytohaemagglutinin-activated PBMC were infected with HIV-1, HHV-6 or both viruses simultaneously. Details of the infection procedure and indirect immunofluorescence assay are given in the legend to Fig. 1.

† Per cent immunofluorescent-positive viable cells and in parenthesis absolute numbers of positive viable cells $\times 10^{-4}$ (averaged results from three separate experiments) for the indicated virus, determined as described in the legend to Fig. 1.

‡ Presence (+) or absence (–) of syncytia (S) as evaluated on Wright–Giemsa stained cytocentrifuge preparations, each containing 2×10^5 cells.

‖ NA, not applicable. In these cultures no viable cells were detected by day 10 post-infection.

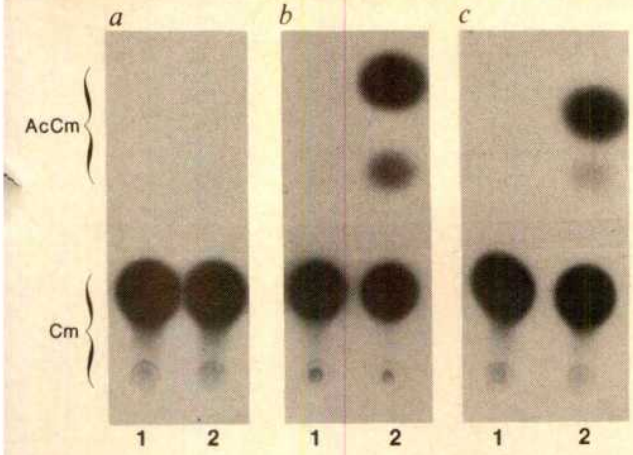


Fig. 5 Transactivation of the choramphenicol acetyltransferase (*CAT*) gene linked to the HIV-1 LTR when transfected into MOLT-3 cells which were uninfected (*a*), infected with HIV-1 (*b*) or infected with HHV-6 (*c*). Plasmids were pC15CAT (lanes 1; ref. 21) containing the HIV-1 LTR linked to the bacterial *CAT* reporter gene, and the control pSVOCAT (lanes 2; ref. 22) that lacked promoter-enhancer sequences.

Methods. 10^7 cells were transfected with 10 μg plasmid DNA by the DEAE-dextran technique[23], collected after 40 h and processed to obtain 100 μl cellular extract; aliquots of 20 μl were subsequently used for CAT assays in a 3-h incubation[24]. Different percentages of cell viability were found in the various cultures at the time of collection: *a*1, 79%; *a*2, 89%; *b*1, 51%; *b*2, 49%; *c*1, 26% and *c*2, 29%. The acetylation of chloramphenicol (%) as estimated from liquid scintillation counting was *a*1, 0.19; *a*2, 0.24; *b*1, 2.5; *b*2, 30; *c*1, 0.27 and *c*2, 19.7. Cm, chloramphenicol; AcCm, acetylated chloramphenicol.

The accelerated virus expression and cytopathicity in dually infected cultures suggested that a direct interaction occurs between HIV-1 and HHV-6. We therefore studied the transactivation of the HIV-1 LTR by HHV-6 using the human $CD4^+$ T-cell line MOLT-3. As illustrated in Fig. 5, there was a consistent transactivation in HHV-6-infected cells which was not found in uninfected cells. We obtained similar results using the $CD4^+$ T-cell line CEM (data not shown).

The part played by cofactorial mechanisms in the pathogenesis of AIDS and in particular the relevance of other potentially immunosuppressive agents are still to be clarified. We have previously demonstrated that HHV-6 exerts a direct lytic effect on human $CD4^+$ T lymphocytes[18]. Here we have shown that HHV-6 can co-infect individual T cells with HIV-1 and can transactivate the expression of HIV-1. The simultaneous presence of both viruses in the same cell emphasizes the potential importance of this transactivation. Replication of HHV-6 might in turn be enhanced by co-infection with HIV-1, thus generating a vicious cycle. Our observations, although they do not prove the aetiological involvement of HHV-6, are consistent with a possible cofactorial role of this virus in one of the critical pathological features of AIDS, namely the $CD4^+$ T-cell depletion. Prospective long-term seroepidemiological and *in vivo* investigations, using animal models or two-probe hybridization to detect dual infection in individual patient cells, will help evaluate the contribution of potential cofactors to the course of HIV-1 infection.

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Genetically haploid spermatids are phenotypically diploid

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Because chromosomal homologues segregate from one another during meiosis, spermatids are genetically different. Post-meiotic gene expression could lead to gametic differences, some of which might lead to preferential transmission of certain alleles over others. In both insects and mammals, however, all the cells derived from a single spermatogonial cell develop within a common syncytium formed as a result of incomplete cytokinesis at each of the mitotic and meiotic cell divisions[1–3]. It has been proposed that the intercellular bridges connecting the cells, which are about 1 μm in diameter[4], permit the sharing of cytoplasmic constituents, thus ensuring the synchronous development of a clone of cells and gametic equivalence between haploid spermatids[2,5,6]. By analysing the product of a transgene which is expressed exclusively in post-meiotic germ cells in hemizygous transgenic mice, we have shown that genetically distinct spermatids share the product of the transgene and hence can be phenotypically equivalent.

The experiments described here were designed to determine whether either RNA or protein can pass through the intercellular bridges which connect spermatids. To address these questions we have generated transgenic mice that carry a chimaeric gene consisting of the mouse protamine 1 (*mP1*) transcriptional regulatory sequences, fused to the human growth hormone (*hGH*) structural gene. The advantage of using transgenic mice for such an analysis is that mice can be generated which are hemizygous for the transgene, that is, the introduced DNA is present on only one of the two chromosomal homologues, so that after meiosis only half of the spermatids carry the transgene (Fig. 1*a*). The *mP1* gene is transcribed exclusively during spermiogenesis, the haploid phase of spermatogenesis[7]. Previous studies have shown that 4.8 kilobase (kb) of 5′ sequence is sufficient for the proper tissue-specific and temporal regulation of *mP1* in transgenic mice[8]. The construct used in this study contains 4.1 kb of *mP1* 5′ sequence fused to the *hGH* gene (Fig. 1*b*).

Two independent lines of transgenic mice harbouring the *mP1-hGH* gene were established (Fig. 1*b*). To determine the transmission frequency of the transgene, tail DNA from

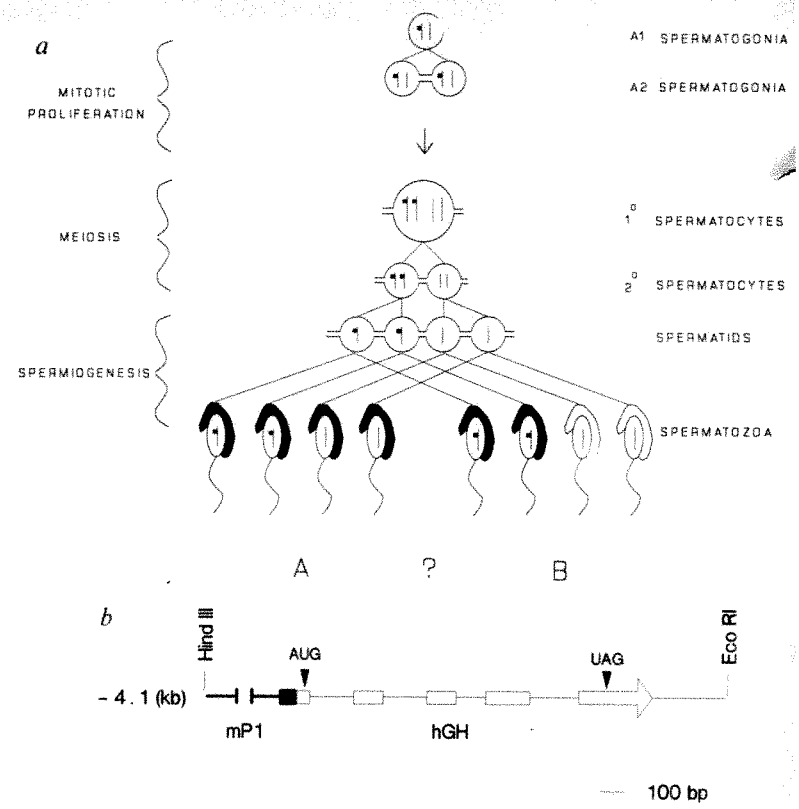


Fig. 1 *a*, Illustration of the syncytial nature of spermatogenesis and the three general stages of development[20,21]. The spermatogonial cells are drawn with a pair of homologous chromosomes inside them with one chromosome containing the introduced transgene (shown by a solid box). After the commitment of A1 spermatogonial cells to differentiate there are five or six rounds of mitotic proliferation. The first cell division generating A2 spermatogonial cells is shown. Primary spermatocytes contain a 4N complement of DNA, that is, each chromosome is associated with a sister chromatid. Each primary spermatocyte gives rise to four spermatids, two that contain a wild-type chromosome and two that carry a chromosome with the transgene. During spermiogenesis, round spermatids are transformed into mature spermatozoa. The question is whether all of the spermatozoa contain the product of the transgene (in this case the hGH protein localized to the acrosome), as shown in A, or whether only those spermatozoa that contain the transgene will have the product, as shown in B. *b*, Plasmid *mP1-hGH* contains approximately 4.1 kb of *mP1* 5′ sequence, including the promoter and 91 base pairs (bp) of 5′ untranslated sequence fused to a genomic clone for the *hGH* gene. Boxes represent exons and lines represent either nontranscribed DNA or introns. Bold lines and the solid box represent *mP1* DNA whereas thin lines and open boxes represent *hGH* DNA. The break in the line that corresponds to *mP1* sequence denotes that portion of the transgene that is not drawn to scale. The positions of the translational start and stop codons in the first and last exon, respectively, are shown above the gene structure. Transgenic mice were constructed by microinjecting a 6.3 kb *Hin*dIII-*Eco*RI fragment containing the *mP1-hGH* gene, free of plasmid vector sequences, into the pronuclei of fertilized C57BL/6×SJL F2 mouse eggs[22]. Two lines of transgenic mice, 314-4 and 315-6, were generated and used for this study. Both lines contain multiple copies of the transgene integrated on one chromosome.

offspring was analysed for the presence of the transgene by hybridization with an *hGH*-specific radiolabelled DNA probe. Analysis of the progeny from three generations of outcrosses of transgenic with nontransgenic animals showed that the mice were hemizygous for the transgene (data not shown).

In situ hybridization was used to determine the developmental stage in which the transgene is first transcribed. Figure 2*a* and *b* shows serial sections of a testis prepared from a hemizygous transgenic adult male, and hybridized with ^{35}S-labelled antisense RNA probes specific for the *mP1-hGH* transgene and the endogenous *mP1* gene, respectively. In both cases expression was detected exclusively in the haploid spermatids and not in spermatogonial cells or in spermatocytes.

Northern blot analysis of RNA isolated from the testis of prepuberal transgenic animals of different ages supported the conclusion that the transgene was expressed exclusively in post-meiotic cells. No transcripts were detected from the transgene or the endogenous gene in testis from 19-day-old animals, an age at which cells in the first cycle of spermatogenesis have progressed to the pachytene spermatocyte stage[9,10], whereas transcripts from both genes were detected, in about equal abundance, in 25-day-old animals, an age at which some cells have progressed into the haploid stage of development (data not shown).

To determine the phenotype of spermatids with respect to the product of the *mP1-hGH* transgene we used immunocytochemical analysis. Spermatozoa from transgenic mice harbouring the *mP1-hGH* construct contain the hGH protein localized in the acrosomal compartment (R.E.B. *et al.*, in preparation). Visualization of the hGH protein in developing acrosomes in early-stage seminiferous tubules (Fig. 3*a*), and in later-stage tubules (Fig. 3*b*), showed that the majority of the spermatids within a tubule of a hemizygous mouse were immunopositive for the hGH protein. Immunocytochemistry performed on the caput epididymis (the proximal portion of the epididymis where newly formed spermatozoa reside), showed that the hGH protein was stable in the acrosome and that, once again, most of the spermatozoa were immunopositive (Fig. 3*c*).

To quantitate the percentage of hGH-immunopositive spermatozoa, we isolated sperm from the caput epididymis (Fig. 3*d*) and determined directly the percentage that were immunopositive. Approximately 90% of all caput sperm analysed from hemizygous animals contained hGH-immunopositive acrosomes (Table 1), despite the fact that the gene was transmitted to only 50% of the offspring, showing that post-meiotic gene products can be shared.

Failure to detect hGH protein in 9% of the spermatozoa could be due to incomplete movement of RNA or protein between spermatids, nondetectable levels of expression of the transgene in some spermatids, or technical problems such as loss of the acrosome during the sperm isolation. To distinguish between incomplete movement of RNA or protein between the intercellular bridges and the other possibilities, we analysed caput sperm from mice homozygous for the transgene. Even in homozygous mice only 92% of the sperm were immunopositive (Table 1),

Table 1 Quantitation of the percentage of hGH-immunopositive spermatozoa

Genotype	Total sperm analysed	Percentage immunopositive
Hemizygous	2,185	91%
Homozygous	2,768	92%

Spermatozoa were isolated from the caput epididymis and immunocytochemistry was performed as described in the fig. 3 legend. These results were obtained by analysing spermatozoa isolated from three hemizygous mice (two from the 315-6 line and one from the 314-4 line) and two homozygous mice (both from the 314-4 line). Homozygous mice were obtained by crossing hemizygous transgenic animals and screening the offspring by quantitative DNA dot hybridization with an *hGH*-specific probe. Cells were counted using differential interference contrast microscopy at ×400 magnification.

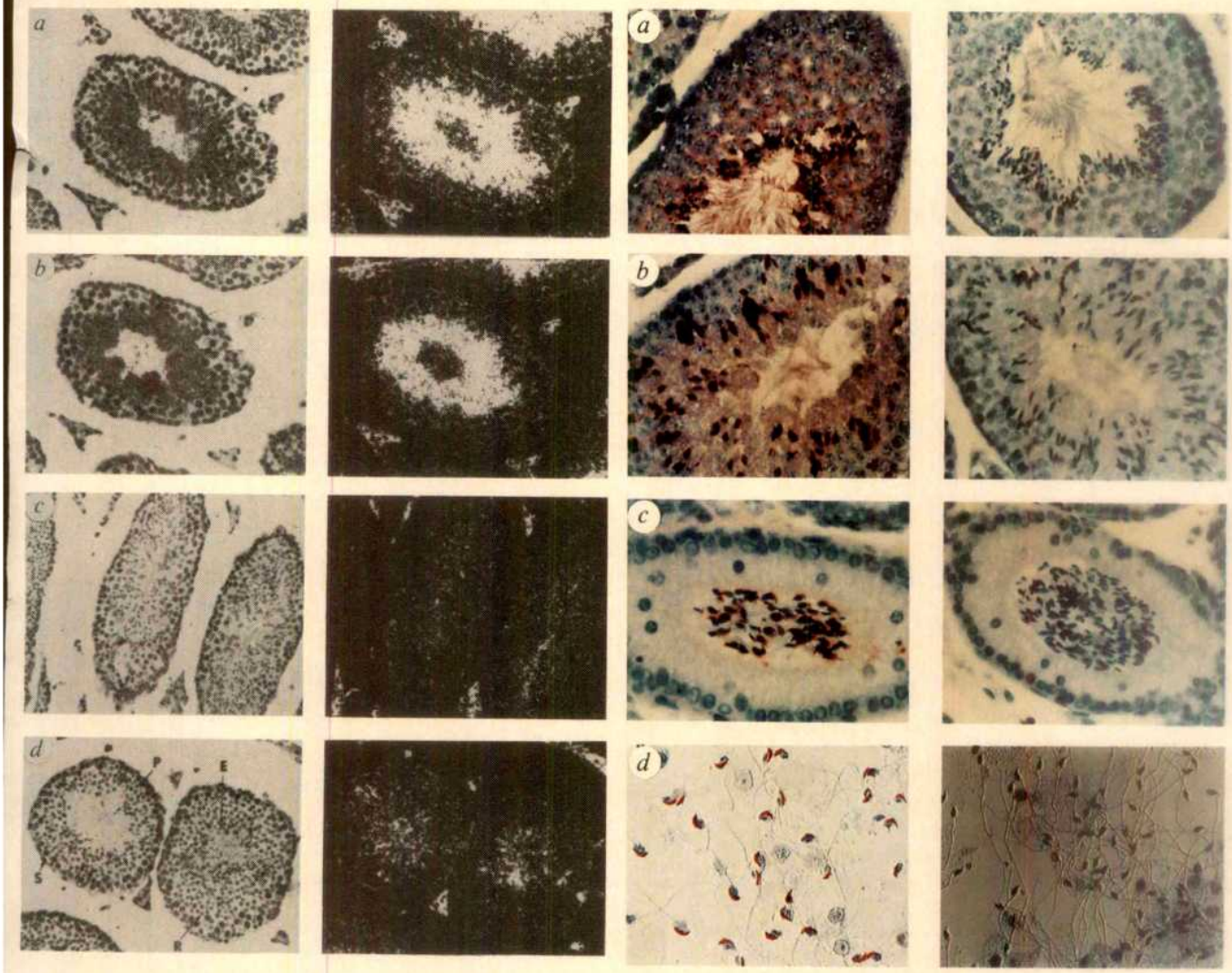


Fig. 2 *In situ* hybridization of testis sections from a transgenic mouse harbouring the *mP1–hGH* gene (line 314-4). Serial sections were hybridized with ^{35}S-labelled probes: *a*, *hGH* antisense RNA; *b*, *mP1* antisense RNA; *c*, *hGH* sense RNA; and *d*, *mP1* sense RNA. Photographs on the left were taken with bright-field microscopy and those on the right with dark-field microscopy. Examples of the different cell types found within a seminiferous tubule are shown by arrows and are labelled as follows: S, spermatogonial cell; P, pachytene spermatocyte; R, round spermatid; and E, elongated spermatid.

Methods. Single-stranded ^{35}S-containing sense and antisense riboprobes were made *in vitro* with linear templates and either SP6 or T7 RNA polymerase. The *mP1* template was made by cloning a 250-bp restriction fragment containing a portion of *mP1* exon 1, the intron, and a portion of exon 2 into pGEM-2. The *hGH* template was constructed by cloning a 170 bp restriction fragment containing *hGH* exon 5 into pGEM-2. Testes were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5 μm sections. Hybridizations were as described[23]. Probes were used at a concentration of about 0.2 ng μl^{-1} with a specific activity of approximately 5×10^5 c.p.m. ng^{-1}. Hybridizations were at 45° overnight. The most stringent washing conditions were at 45° in 0.1 × SSC. Slides were developed after one to three days. Sections were counterstained with haematoxylin.

suggesting that it is not incomplete sharing of post-meiotic gene products that leads to the immunonegative cells.

Development of the acrosome occurs adjacent to the nucleus during spermiogenesis[9,11,12]. Juxtaposed to the acrosome is the cellular machinery responsible for synthesizing the acrosomal enzymes, the Golgi apparatus and the rough endoplasmic reticulum (RER)[12,13]. Because hGH protein is localized to the

Fig. 3 Immunocytochemistry of hGH protein in *mP1–hGH* transgenic mice (line 314-4). Photographs on the left show sections incubated in the presence of both the primary antibody (rabbit anti-hGH), and secondary antibody (biotinylated goat anti-rabbit IgG). Photographs on the right show sections incubated only in the presence of the secondary antibody. *a*, An early-stage seminiferous tubule with round spermatids at the CAP phase of acrosome development[9]. *b*, A late-stage tubule with many elongated spermatids and fully developed acrosomes. *c*, A section through the caput epididymis. *d*, Spermatozoa isolated from the caput epididymis. The hGH protein is seen as a red precipitate.

Methods. Testes were fixed in Carnoy's, embedded in paraffin and cut into 5 μm sections. Sections were deparaffinized with xylene and re-hydrated using standard procedures. Indirect immunocytochemistry was performed using the reagents and suggested protocols of Vectastain ABC and Zymed SABC kits. The primary antibody, rabbit anti-hGH, was obtained from the National Hormone and Pituitary Program, Baltimore, Maryland, and used at either a dilution of 1/100 or 1/500. Aminoethyl carbazole (Zymed Lab. Inc.) was used as the substrate chromogen. Sections were counterstained with haematoxylin. Additional negative controls included the use of both the primary and secondary antibodies on testes isolated from nontransgenic animals. For the isolated epididymal sperm, transgenic animals were killed, the caput epididymis dissected out and cut into 8–10 pieces, and incubated for 30 min at 30 °C in EKRB media[24]. After 30 min the supernatant was removed, centrifuged for 5 min at 1,000 r.p.m., and the pellet resuspended in a small volume of EKRB media. A few μl of sample were spread onto a microscope slide, allowed to dry for about 1 min, and fixed in Carnoy's. After incubation in PBS for 30 min, immunocytochemical analysis was performed as described above.

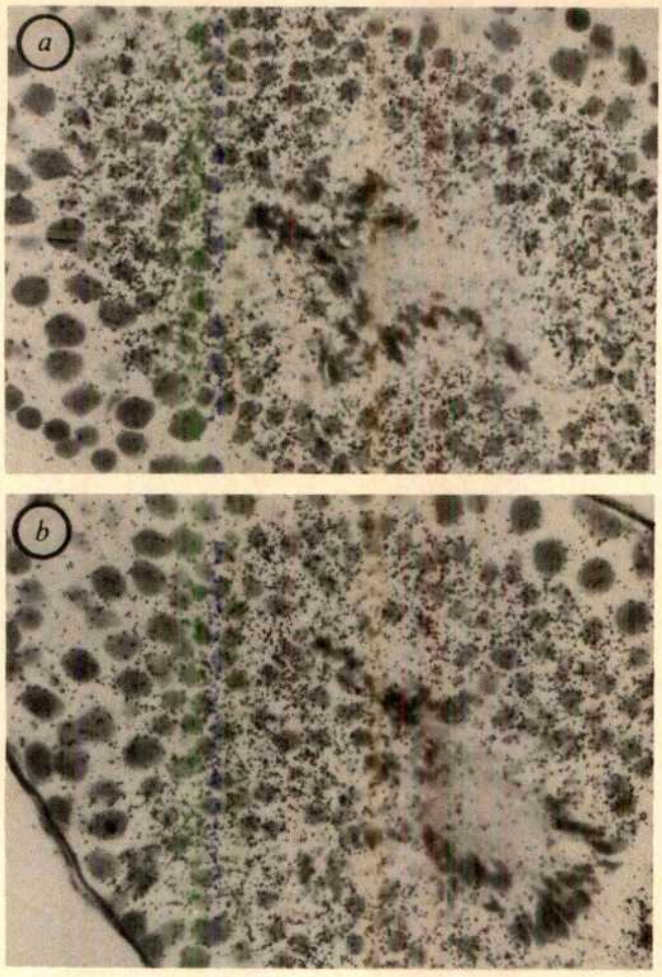


Fig. 4 *In situ* hybridization of testis serial sections hybridized with either an *hGH* antisense probe (*a*) or an *mP1* antisense probe (*b*). Sections were photographed at ×500 magnification. See Fig. 2 legend for details of hybridization conditions.

acrosome, it is probably synthesized on the RER, at the site of acrosome formation, and is therefore not free in the cytoplasm[14]. We first detect hGH immunocytochemically in the developing acrosome (Fig. 3*a*). Although it is unlikely that vesicles bud off from the Golgi and move between the syncytial bridges, electron microscopy studies have shown that the endoplasmic reticulum does sometimes extend through the bridges[15] suggesting that growth hormone protein could be traversing the bridges through the ER. Comparison of serial sections hybridized with *hGH*-specific and *mP1*-specific RNA probes (Fig. 4*a*, *b*), however, showed that within a seminiferous tubule there seemed to be an equal distribution of silver grains over all of the round spermatids regardless of the probe used. These results suggest that there could be movement of *mP1-hGH* mRNA, presumably through the intercellular syncytial bridges. Experiments designed to test unequivocally for movement of mRNA are currently in progress.

These results provide evidence against functional differences between sperm arising from post-meiotic gene expression. Our results are supported genetically, with one exception, by the absence of any cases of non-mendelian inheritance in the mouse[16]. The one exception involves preferential transmission of the t locus in male mice heterozygous for the t chromosome[17]. Male mice heterozygous for a complete *t*-haplotype can transmit the t-haplotype chromosome with a frequency as high as 99%[18]. Although transmission ratio distortion in *t*-haplotypes might be due to preferential retention of mRNA and/or protein in spermatids in which they are synthesized[16,19], it has yet to be demonstrated that post-meiotic transcription is definitely involved in the transmission ratio distortion. Our data do, however, clearly show that, at least for the product of the *mP1-hGH* transgene, either mRNA and/or protein is free to move between the intercellular bridges which connect developing spermatids, and suggest that the products of most genes may be equally distributed among spermatids.

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Tnt1, a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics

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Transposable elements can be identified by their ability to induce mutant alleles at new loci. The retrotransposon family is thought to transpose through an RNA intermediate and has many similarities to vertebrate proretroviruses[1,2]. In plants, retrotransposons have been described in maize[3,4], *Arabidopsis*[5] and wheat[6], and non-viral retroposons in maize[7]. Most of these elements, however, have been found as non-mobile integrated units. Here, we report the isolation of the first tobacco (*Nicotiana tabacum*) transposable element, Tnt1, which seems to be the most complete mobile retrotransposon characterized in higher plants. Tnt1 has been isolated after its transposition into the nitrate reductase (NR) structural gene of tobacco, and transposition events have been detected through *in vitro* selection of spontaneous NR-deficient (NR^-) mutant lines in cell cultures derived from tobacco mesophyll protoplasts. Tnt1 is 5,334 nucleotides long, contains two 610-base-pair-long terminal repeats and a single open reading frame of 3,984 nucleotides. Comparison of the Tnt1 open reading frame coding potential with those of the *Drosophila melanogaster copia*

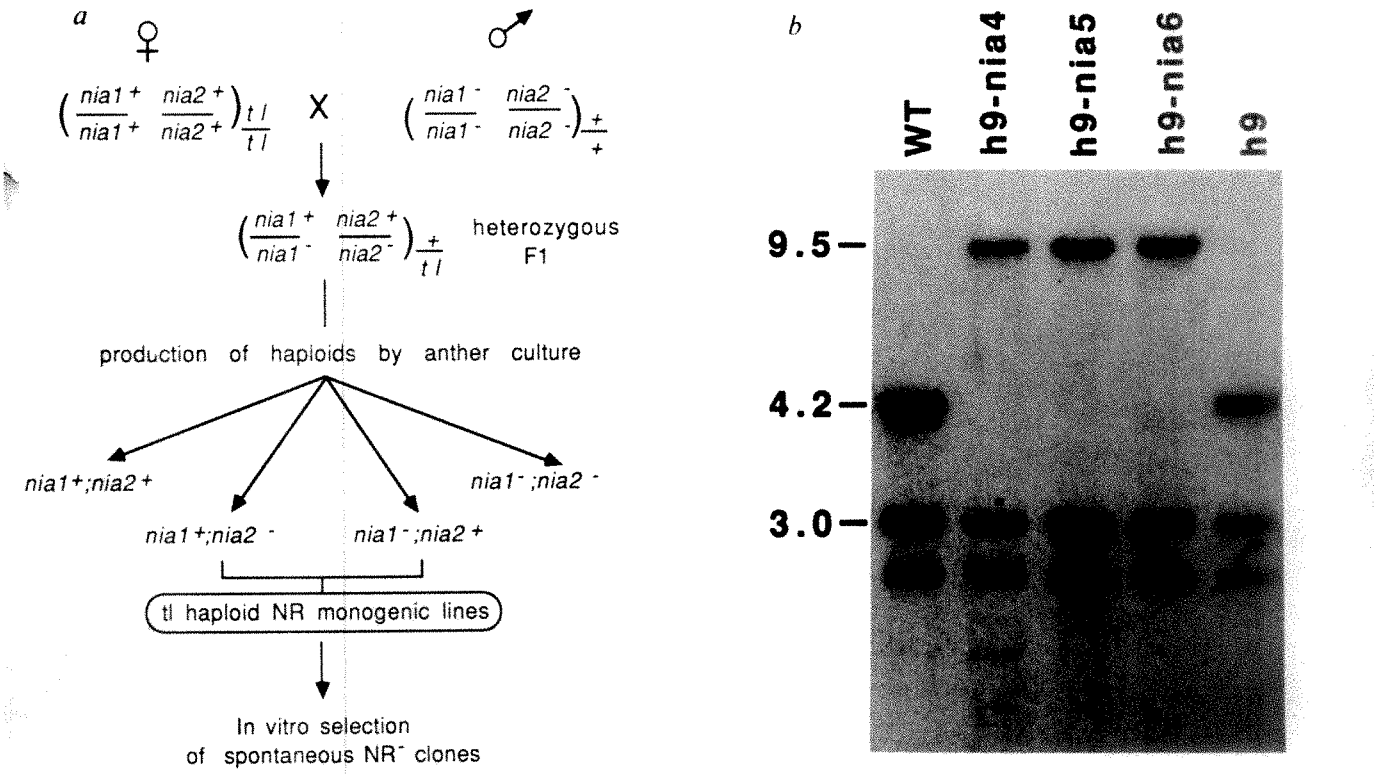


Fig. 1 Isolation and characterization of three spontaneous NR⁻ mutants induced by transposition of Tnt1 into the coding sequence of the *nia2* locus. *a*, The construction of haploid monogenic lines from which spontaneous NR⁻ clones were selected. Stably mutated *nia* alloalleles from a NR⁻ (*nia1*⁻; *nia2*⁻) tobacco line[12] were introduced into a NR⁺ (*nia1*⁺; *nia2*⁺) tl line by crossing and subsequent anther culture[29] of the F1. Haploid NR monogenic tl lines (*nia1*⁺; *nia2*⁻) or (*nia1*⁻; *nia2*⁺) were screened by genetic tests on derived diploidized plants[29]. Mesophyll protoplasts were isolated[30] from haploid lines and spontaneous chlorate-resistant clones were selected on derived colonies[13]. The NR deficiency of these clones was confirmed by their inability to grow on nitrate as the sole nitrogen source. NR⁻ plants were regenerated and propagated in the greenhouse by grafting[31]. *b*, Southern blot analysis of *Eco*RI restriction digests of total DNA isolated from three independent NR⁻ mutants selected in cell cultures from the h9 (*nia1*⁻; *nia2*⁺) tl monogenic line. WT = wild-type XHFD8 tobacco line[29]. Structure of the stably mutated *nia* genes originating from the NR⁻ line[11] is similar to the wild-type *nia* genes[14]. The probe was a 1.6 kb *Eco*RI NR cDNA fragment which hybridizes similarly to both alloalleles, revealing a 3 kb band from the wild-type *nia1* gene and a 4.2 kb band from the wild-type *nia2* gene. An additional 2.8 kb band shows homology to the probe, but is not correlated to the *nia1* or *nia2* structural genes[14]. In the three h9-Nia4, h9-Nia5 and h9-Nia6 mutants, the *nia2* 4.2 kb *Eco*RI fragment has disappeared and a new 9.5 kb band is present. Sizes are in kb.

Methods. Total plant DNA was isolated by a modified Dellaporta procedure[32] and purified on a CsCl gradient. DNA (5–6 μg per lane) was digested and Southern blot analysis was performed as previously described[11].

retrotransposon[8], yeast Ty retrotransposon[9], and vertebrate pro-retroviruses[2] revealed that Tnt1 is closely related to *copia* and carries all the functions known to be required for autonomous transposition by reverse transcription.

The primary aim of this work was the isolation of a transposable element thought to be responsible for a chlorophyllian somatic instability in a mutant tobacco line named tl (ref. 10). We used cell genetic techniques to detect the transposition of this element into the NR structural gene, for which a cDNA molecular probe was available[11]. Tobacco is an amphidiploid species arising from the combination of two diploid genomes of wild-type *Nicotianeae* species. To induce nitrate reductase deficiency, two functional homoeologous genes (*nia1* and *nia2*) encoding the apoenzyme of nitrate reductase have to be simultaneously mutated in the haploid tobacco genome[12]. Haploid NR monogenic tl lines were constructed, in which one of the two *nia* alloalleles is stably mutated, and in which a single transposition event can induce a NR⁻ phenotype (see Fig. 1*a*). Spontaneous NR⁻ clones were selected from mesophyll protoplast-derived cells by a direct selection for resistance to chlorate[13]. Several spontaneous NR⁻ clones were isolated at frequencies of up to 10^{-4} and regenerated into plants. Molecular analysis of regenerated NR⁻ plants showed that this phenotype was associated, in one-third of the clones analysed, with a modification of the restriction pattern of the wild-type *nia* alloallele. In three independent NR⁻ mutants selected from the h9 NR monogenic line (*nia1*⁻; *nia2*⁺), the 4.2 kilobase (kb) *Eco*RI fragment present within the wild-type *nia2* gene[14] has disappeared and a new hybridizing band of about 9.5 kb is present (Fig. 1*b*). More detailed restriction mapping revealed that an insertion of 5.3 kb was located into the first exon of the *nia2* gene in mutant h9-Nia6, the second in mutant h9-Nia5, and the third exon in mutant h9-Nia4 (data not shown).

To isolate the 9.5 kb *Eco*RI band, partial genomic libraries were constructed in phage λ NM1149 (ref. 15) from mutants h9-Nia4 and h9-Nia5, and screened with the NR cDNA probe. Clone LNM94 was isolated from the h9-Nia4 library and clone LNM95 was isolated from the h9-Nia5 library. In both clones, a 5.3 kb insertion is found at the expected location in the *nia2* gene. The structure of the *nia* regions adjacent to the insertion is similar to the structure of the wild-type gene, indicating that no other DNA rearrangement has occurred in the two mutants. Both insertions cross-hybridize and have similar restriction maps (Fig. 2), indicating that the NR⁻ phenotype is due to the transposition of the same mobile element into the *nia2* gene. The element is, however, inserted in opposite orientation in each mutant. This transposable element has been named Tnt1 (for transposable element of *Nicotiana tabacum*).

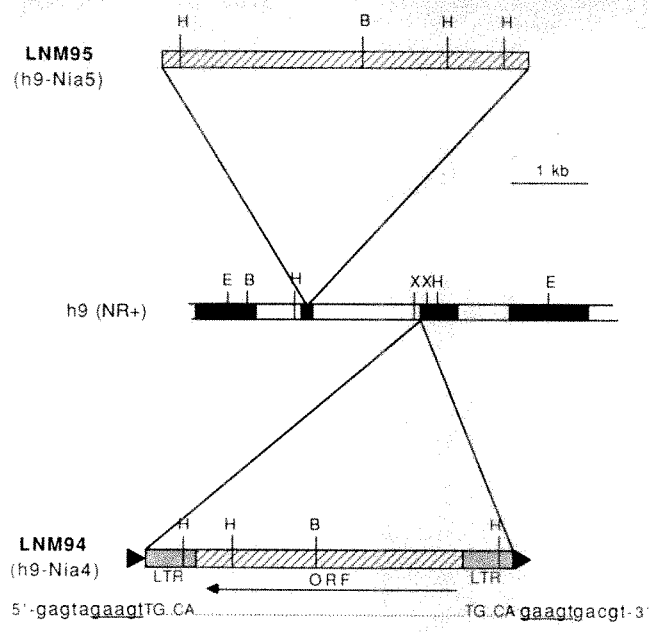


Fig. 2. Restriction maps of the two Tnt1 insertions found in clones LNM94 and LNM95. A restriction map of the NR^+ (h9) *nia2* *Eco*RI fragment is shown for reference. Some structural features of the Tnt1 element of LNM94 are shown. Dark boxes represent the coding sequence of the *nia2* gene, hatched boxes the Tnt1 insertion, and shaded boxes the LTRs. Arrow indicates the Tnt1 ORF orientation. The sequence of LTR terminal inverted repeats is represented in capital letters, the sequence of the *nia2* gene[14] in lower-case letters, and the sequence of the target site duplications (dark triangles) is underlined. B = *Bam*HI, E = *Eco*RI, H = *Hind*III and X = *Xho*1.

Methods. Plant DNA (150 μg) was digested by *Eco*RI and fractionated on sucrose gradient as described[33]. Fractions containing the 9.5 kb band hybridizing to the NR cDNA probe were purified and ligated to the insertion vector λ NM1149 (ref. 15). Because of the high instability of the Tnt1 insertion, further purifications of positive recombinant clones, and subcloning in pBluescript (Stratagene) were performed on $recA^-$ host strains.

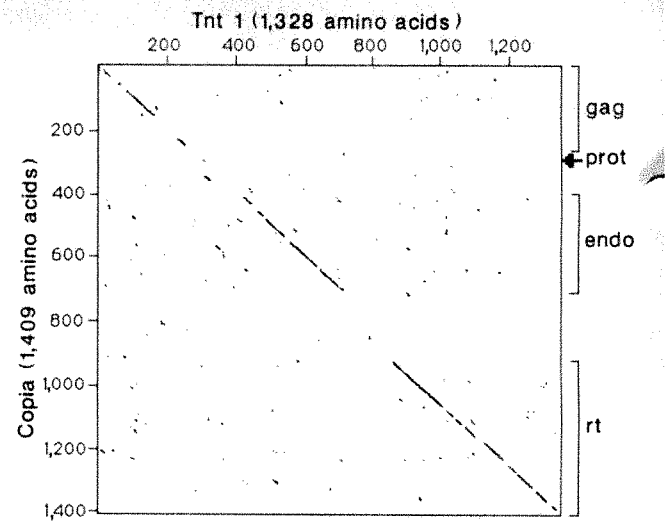


Fig. 3 Homology matrix comparisons between amino-acid sequences of the Tnt1 and the *copia* ORFs. A computer program[34] was used to generate diagonal lines indicating segments of 20 residues that show similarity above a threshold level. Residue numbers are indicated. The approximate regions corresponding to *gag*, and the endonuclease (endo) and reverse transcriptase (rt) domains of the *pol* gene are shown. The active site of the protease (prot) is indicated by an arrow. Respective positioning of all these regions are similar in Tnt1 and *copia*. In proretroviruses and retroviral-like elements, the *gag* gene encodes for the structural core proteins, and the polyprotein encoded by the *pol* gene is maturated by cleavage in several functional units, which are mainly the protease, the endonuclease and the reverse transcriptase. No homology to the *env* region of retroviruses has been found in *copia* and Tnt1.

Methods. Average score values were calculated for pairs of segments of 20 residues, whose amino-acid sequences were compared between Tnt1 and *copia*. If the average score value is greater than 238, a diagonal line is generated on the corresponding position of the homology matrix. This threshold value is the degree of similarity that would occur by chance with a probability of less than 2×10^{-4}. All the computer analyses were performed using the programs available from the Centre Interuniversitaire de traitement de l'Information (CITI 2, Faculté de Médecine, Paris).

The complete sequence of the Tnt1 element found in the mutant h9-Nia4 was determined by the dideoxynucleotide method[16]. Tnt1 is 5,334 base pairs (bp) long and is flanked by a duplication of a short region of host DNA (5 bp) at the integration site. Tnt1 has structures typical of transposable elements of the retrotransposon family (Fig. 2). It is bounded by two identical long terminal direct repeats (LTRs) of 610 bp, which are terminated by the consensus sequence 5'TG . . . CA-3'. Moreover, Tnt1 internal features include most of the structural and functional regions commonly found in retrotransposons and retroviruses. Sequence analysis of the 4,115 bp internal domain of Tnt1 has revealed a single large open reading frame (ORF) of 1,328 amino acids, oriented in the opposite direction to the *nia* coding sequence in the h9-Nia4 mutant. The putative protein encoded by the Tnt1 ORF was compared with retroviral gene products[17-19] and the putative protein encoded by *copia* ORF[8]. The general organization of the Tnt1 ORF is very similar to the one in *copia*, both in overall structure (*gag-pol*) and in length (1,328 amino acids in Tnt1, 1,409 in *copia*). At the amino-acid level also, a high degree of homology was found between the putative polyproteins encoded by the ORFs of these two elements (Fig. 3): 29% homology for the *gag* polyprotein product (residues 1-265), 42% homology for the endonuclease domain of the *pol* polyprotein product (residues 420-720), and 38% homology for the reverse transcriptase domain of the *pol* polyprotein product (residues 810-1,328 for Tnt1 and 890-1,404 for *copia*). Similarities include several short stretches of amino acids that are highly conserved in all proretroviruses and retrotransposons, which are summarized in Fig. 4. The *gag* regions of *copia* and Tnt1 contain a 14-amino-acid domain located at exactly the same position in both ORFs (amino acids 232-245), which is highly conserved among retroviral nucleic acid-binding proteins[20]. These proteins are required for accurate positioning of the tRNA primer on the replication initiation site at the 5' end of the RNA genome[21]. In most retroviruses and retrotransposons, the 5' end of the *pol* gene codes for a protease involved in the processing of polyproteins. The conserved sequence Asp-Thr(Ser)-Gly is replaced in Tnt1 by the Asp-Thr-Ala sequence, which presumably could also act as an active site of the protease[22]. Moreover, this sequence is found at comparable locations in Rous sarcoma virus (RSV), *copia* and Tnt1 elements, and the relative spacing between this site and the nucleic acid-binding domain is similar in the three genomes. Regions of highest homology between the Tnt1 ORF and *copia* also include conserved domains identified among a wide variety of reverse transcriptase and polymerase gene products[23-25]. One of the most highly conserved domains was found at amino acids 1,005-1,014 of the Tnt1 ORF (amino acids 1,012-1,021 of the *copia* ORF). This region (Tyr-Met or Val-Asp-Asp followed by strictly hydrophobic long-chain amino acids) is found not only in reverse transcriptases, but also in RNA-templated viruses[26]. The 3' region of the *pol* gene of a variety of vertebrate retroviruses also encodes an endonuclease[1,23]. In Ty and *copia*, there is homology to this region at the 5' end of the *pol* gene[8], as in Tnt1.

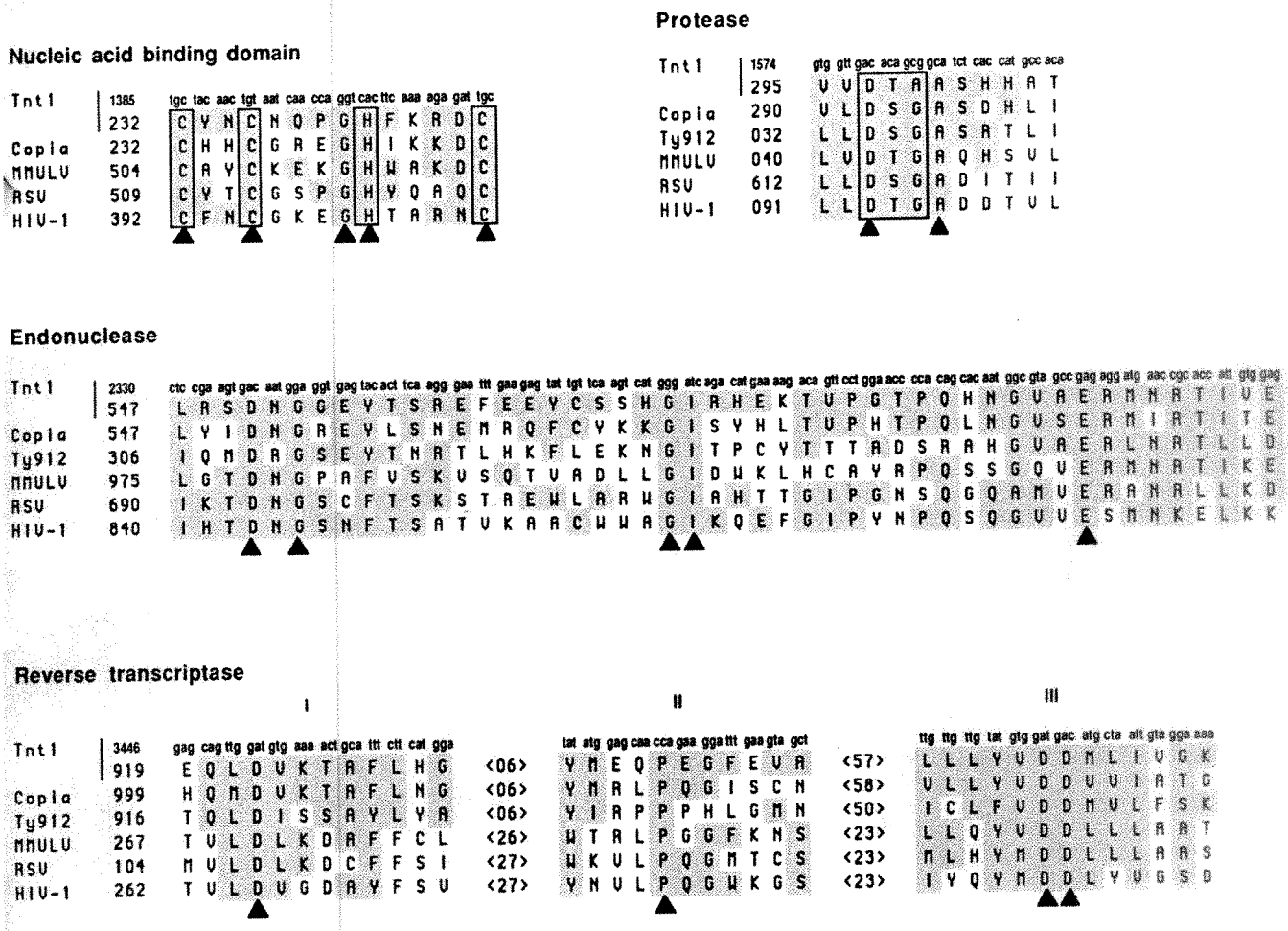


Fig. 4 Comparison of the Tnt1 ORF with conserved domains found in retroviruses and retrotransposons. Amino-acid sequences are indicated in capital letters, and the corresponding nucleotide sequence for Tnt1 is indicated in lower-case letters. Conserved and nearly invariant amino acids are shaded. Fully conserved positions are indicated by arrows. *Copia* is a retrotransposon from *Drosophila melanogaster*[8]; Ty-912 is a member of the retrotransposon family Ty1 in *Saccharomyces cerevisiae*[9]; MMULV is the Moloney murine leukemia virus[17]; RSV is the Rous sarcoma virus[18]; HIV-1 is the human immunodeficiency virus[19]. Alignments are those previously published[23–25]. Nucleic acid-binding domain[20]: the cysteine and histidine residues involved in the zinc-binding domain are boxed. Protease domain[22]: the conserved D-T-(S)-G amino-acid sequence is boxed and numbers indicate amino-acid positions, starting from *gag* (Tnt1, *copia* and RSV), *pol* (MMULV, HIV-1) or ORFb (Ty-912). In the endonuclease domain[23], only the most highly conserved domain is represented. In the reverse transcriptase domain, the three motifs are compared as in ref. 25 and the length separating the motifs is indicated between brackets.

From these sequence comparisons, we conclude that the Tnt1 ORF encodes a polyprotein containing the necessary functions for its autonomous transposition by reverse transcription of an RNA intermediate: the gag core proteins[2], a protease[22], a reverse transcriptase and an endonuclease[1,23]. The high degree of amino-acid sequence conservation over the entire length of Tnt1 and *copia* ORFs indicate that Tnt1 is highly related to *copia*, and suggests that they might share a common ancestral origin, raising the question of possible horizontal transmission of such elements between insects and plants.

Whether Tnt1 is active in the plant throughout the life cycle, or only at defined stages of development, is not known. Tnt1 might have been activated by tissue culture, as reported for Ac in maize[27]. Experiments are in progress to detect RNA species related to Tnt1, as well as potential hybrid transcripts of the *nia2* gene containing Tnt1 insertions.

The presence of Tnt1 in wild-type tobacco and several other plant species was investigated by Southern blot analysis with a probe specific to Tnt1 (data not shown). This element is present at very high copy number (at least 100 copies per haploid genome) in wild-type and tl tobacco, and in *Nicotiana plumbaginifolia*. Cross-hybridization was also found in other *Solanaceae* species (*Petunia hybrida*, *Solanum tuberosum*, *Lycopersicon esculentum*). These results suggest that Tnt1-related sequences were present in the *Solanaceae* family during the very early stages of speciation. No cross-hybridization could be detected with a number of other dicotyledon and monocotyledon species (*Arabidopsis thaliana*, *Sinapis alba*, *Beta vulgaris*, *Cucumis sativus*, *Trifolium repens*, *Medicago sativa*, *Pisum sativum* and *Zea mays*).

The relationship between the high somatic instability of the tl mutation and the activity of Tnt1 is hypothetical. Retrotransposons are known to excise at very low frequencies, by recombination between LTRs. It is therefore possible that Tnt1 is not involved in the tl unstable mutation. In maize, however, the Adh1-S5446 mutation caused by insertion of Bs1 has been described as highly unstable[28].

No previous example of the use of transposable elements for the *in vitro* selection of biochemical mutants at the somatic level has yet been reported in plants. Until now, gene tagging in plants has required screening of large populations of plants for visible mutation. If suitable cellular systems are available, new ranges of mutations can be obtained by selection at the cell level, and the type of approach described here could therefore extend the potential of transposable elements as genetic engineering tools in eukaryotes, especially in plants.

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Note added in proof: A quick analysis of the amino-acid sequence homologies between Tnt1 and the Ta1 retrotransposon recently isolated in *Arabidopsis*[5] has given the following approximations: endonuclease (integrase) domain, 42%; reverse transcriptase domain, 52% (D. Voytas, personal communication). Both elements seem therefore to be more closely related to each other than to *copia*.

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The relationship of a prochlorophyte *Prochlorothrix hollandica* to green chloroplasts

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It is generally accepted that chloroplasts arose from one or more endosymbiotic events between an ancestral cyanobacterium and a eukaryote[1]. Such an origin fits well in the case of the chloroplasts of rhodophytes that, like cyanobacteria, contain chlorophyll *a* and phycobilin pigments[2]. The green chloroplasts from higher plants, green algae, and euglenoids however, contain chlorophyll *b* as well as chlorophyll *a*, and lack phycobilins. Consequently, it has been suggested that they arose independently of the rhodophyte chloroplasts, from an ancestral prokaryote containing that complement of pigments[3]. The 'prochlorophytes' *Prochloron didemni* (an exosymbiont on didemnid ascidians[4,5]) and *Prochlorothrix hollandica* (a recently discovered, free-living, filamentous form[6]) have been suggested to be modern counterparts of the ancestor of the green chloroplasts because they are prokaryotes that also contain both chlorophylls *a* and *b*, and lack phycobilins[7,8]. We report here a 16S rRNA-based phylogenetic analysis of *P. hollandica*. The organism is found to fall within the cyanobacterial line of descent, as do the green chloroplasts, but it is not a specific relative of green chloroplasts. Thus, similar pigment compositions do not necessarily reflect close evolutionary relationships.

Analysis of RNase T1-generated oligonucleotide catalogues of 16S rRNA from several samples of *Prochloron* sp. indicated that this symbiont falls within the cyanobacterial line of descent, but it seemed to be more closely related to the filamentous, heterocystous cyanobacteria of the genera *Nostoc* and *Fischerella* than to green chloroplasts[9,10]. This interpretation of the data has been disputed for methodological reasons[11]. Although the inability to cultivate *Prochloron* sp.[12] has retarded the investigation of its biochemistry and molecular biology, the discovery of *P. hollandica*[6] provided a tractable model for detailed investigations of prochlorophytes. The presence of chlorophyll *b* in *P. hollandica* suggests that it might be the closest known, free-living relative to the green chloroplasts.

At present, the best method for evaluating evolutionary relationships is the comparison of homologous macromolecular sequences. The 16S rRNA was the natural choice for the phylogenetic analysis of *P. hollandica* because of the broad database available, of cyanobacterial and chloroplast 16S rRNA sequences[13]. About 1,200 nucleotides of the *P. hollandica* 16S rRNA sequence were determined by dideoxynucleotide termina-

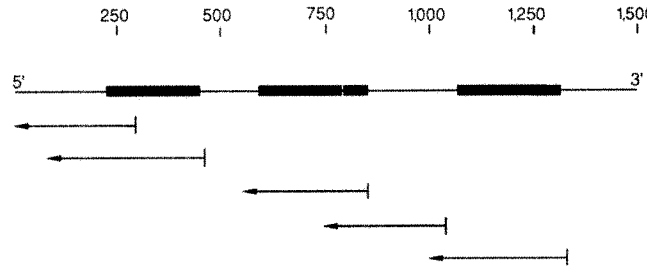


Fig. 1 Regions of 16S rRNA sequenced. *Prochlorothrix hollandica* cells were grown in liquid batch culture using medium BG-11[26]. Ribosomal RNA was obtained and purified as described[13], except that after phenol extraction and ethanol precipitation of the cell lysate, large rRNA was purified by electrophoresis on a denaturing 2.5% polyacrylamide gel. The band containing 16S RNA was excised from the gel and the RNA was electroeluted into 3M NaOAc (*p*H 5.5), followed by two rounds of ethanol precipitation. Dideoxynucleotide-terminated sequencing of 16S rRNA using reverse transcriptase and synthetic oligodeoxynucleotide primers was as described[13,27]. The *P. hollandica* 16S rRNA is represented as a line segment about 1,500 nucleotides in length. Arrows designate those regions of sequence determined from the the use of individual oligodeoxynucleotide primers. Thickened regions of the line segment represent the nucleotides used in the sequence analysis.

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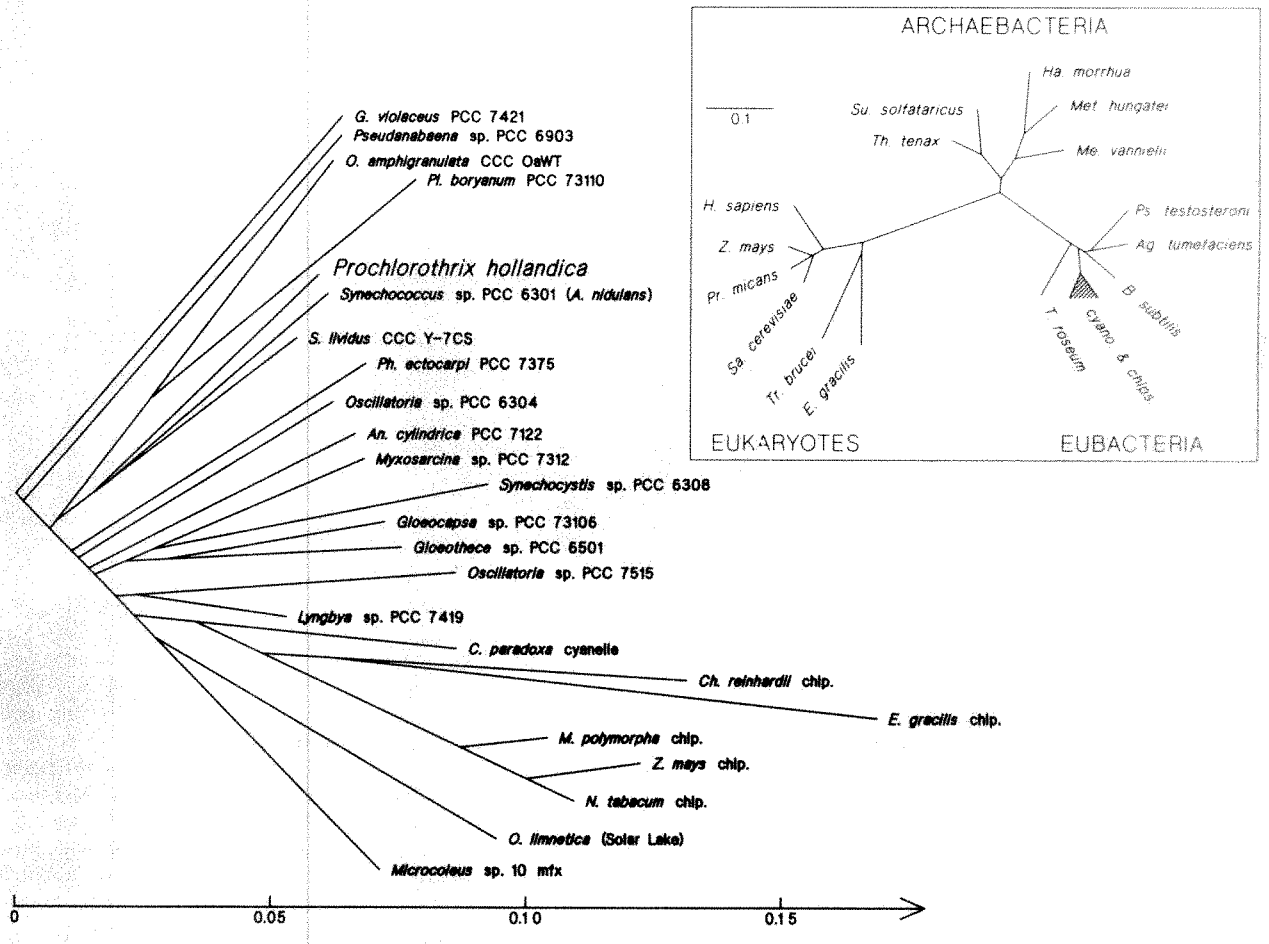


Fig. 2 Rooted phylogenetic tree depicting evolutionary relationships among 16S rRNAs from *P. hollandica*, cyanobacteria, green chloroplasts, and a cyanelle. 16S rRNA sequences from the outgroup organisms *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Pseudomonas testosteroni* were used to locate the root[13]. In this representation, evolutionary distances are proportional to only the horizontal component of the segment lengths. The scale is in units of fixed point mutations per sequence position. Sequence analysis was carried out by a distance matrix method[15,17,28]. Inset: unrooted phylogenetic tree depicting the three primary kingdoms and the relation of the cyanobacteria and chloroplasts to some other eubacteria. Segment lengths are proportional to evolutionary distances. The scale bar represents 0.1 fixed point mutations per sequence position. Adapted from ref. 13. Abbreviations: A., *Anacystis*; Ag., *Agrobacterium*; An., *Anabaena*; B., *Bacillus*; C., *Cyanophora*; CCC, Castenholz Culture Collection; Ch., *Chlamydomonas*; chlp(s)., chloroplast(s); cyano., cyanobacteria; E., *Euglena*; G., *Gloeobacter*; H., *Homo*; Ha., *Halococcus*; M., *Marchantia*; Me., *Methanococcus*; Met., *Methanospirillum*; N., *Nicotiana*; O., *Oscillatoria*; PCC, Pasteur Culture Collection; Ph., *Phormidium*; Pl., *Plectonema*; Pr., *Prorocentrum*; Ps., *Pseudomonas*; S., *Synechococcus*; Sa., *Saccharomyces*; Su., *Sulfolobus*; T., Thermomicrobium; Th., *Thermoproteus*; Tr., *Trypanosoma*; Z., *Zea*.

tion, using reverse transcriptase elongation from oligodeoxynucleotide primers on purified 16S rRNA (Fig. 1). The sequence has been depositied with GenBank and is available from the authors upon request. The inference of the evolutionary relationship of *P. hollandica* to other organisms used a distance matrix method to analyse 677 sequence positions that are approximately equally distributed among the three major domains of the 16S rRNA secondary structure[14] and are unequivocally homologous in all the organisms. The results are presented as a phylogenetic tree in Fig. 2.

P. hollandica is a deeply branching member of the cyanobacteria, most closely related to two unicellular forms, *Synechococcus* sp. strain PCC 6301 (*Anacystis nidulans*) and *Synechococcus* '*lividus*' strain CCC Y7C-S. *P. hollandica* is not specifically related, to the exclusion of other cyanobacteria, to the coherent phylogenetic grouping of the green chloroplasts and the cyanelle of *Cyanophora paradoxa*[13]. We feel that the continuous sequences of the cyanobacterial 16S rRNAs[13] do not overlap sufficiently with the RNase T1-generated, 16S rRNA oligonucleotide catalogues of *Prochloron didemni*[9] and the chloroplast of the red alga *Porphyridium* sp.[15] to allow the reliable inference of a phylogenetic tree that includes these latter components.

The assumption that all sequence positions are equally subject to change can lead to significant errors in the estimation of sequence divergence[16]. Although the likelihood of such errors decreases when sequences of closely related organisms are compared, the possibility of such an occurrence in our analysis was checked by inferring phylogenetic trees under the assumption that the rates of change of sequence positions are described by a log-normal distribution[17]. No differences in tree topology were found.

The reliabilities of several features of the inferred phylogeny were examined by a 'bootstrap' analysis of the data in which sequence positions were randomly selected, with replacement, to produce data sets that were analysed in the same manner as the original data set[18]. Twenty-five 'bootstrapped' phylogenetic trees were generated for this analysis. In 19 of them (76%), *P. hollandica* formed a coherent subgroup with *Synechococcus* sp. strain PCC 6301 and/or *Synechococcus* '*lividus*' strain CCC Y7C-S to the exclusion of all other members of the tree. In 23 'bootstrapped' trees (92%), *P. hollandica* formed coherent subgroups with one or both *Synechococcus* spp. and/or *Phormidium ectocarpi* strain PCC 7375. The association of the cyanelle of *Cyanophora paradoxa* with the green chloroplasts to form a coherent, exclusionary subgroup occurred in 22 'bootstrapped' trees (88%). In no instance did *P. hollandica* group

with one or more of the green chloroplasts or cyanelle to the exclusion of other organisms in the tree. This analysis firmly supports the conclusion that *P. hollandica* is not a specific relative of the green chloroplasts.

On the basis of these rRNA sequence comparisons, the closest known relative of green chloroplasts is the cyanelle of *C. paradoxa*. Cyanelles are believed to be the result of a relatively recent endosymbiosis between a cyanobacterium and a eukaryote[19]. This hypothesis is supported by the fact that cyanelles have both cyanobacterial and chloroplast-like features. Cyanobacterial qualities of cyanelles include a rudimentary peptidoglycan wall, and the presence of chlorophyll *a* and phycobilins. Cyanelles lack chlorophyll *b* and the membrane arrangements that are characteristic of green chloroplasts[19]. The chloroplast-like features of cyanelles include small genome size and genome organization[20,21]. Furthermore, as with chloroplasts, the expression of many cyanelle proteins is under the control of the eukaryotic host[22].

Some lineages that are not known to produce chlorophyll *b* diverged from the main cyanobacterial line at positions intermediate between the lines leading to the green chloroplasts and to *P. hollandica* (Fig. 2). This result implies that the ability to synthesize chlorophyll *b* arose independently in the ancestors of *P. hollandica* and the green chloroplasts, or that it was acquired by lateral transfer. Chlorophylls *a* and *b* differ in a minor way: the oxidation state of a single carbon (methyl in chlorophyll *a* but aldehyde in chlorophyll *b*) so the acquisition of the ability to synthesize chlorophyll *b* would not seem to be a significant biochemical change. A less likely interpretation of the phylogenetic relationships is that *P. hollandica* and the progenitor(s) of green chloroplasts shared an ancestor that produced chlorophyll *b*, but that multiple losses of the ability to synthesize chlorophyll *b* occurred among the intermediate cyanobacterial sublines. In any case, however, we must conclude that similar pigment compositions do not necessarily reflect particularly close evolutionary relationships.

All the currently available data indicate that cyanobacteria, prochlorophytes, and chloroplasts constitute a 'holophyletic' group; that is, a group that consists of a common ancestor and all lineages derived from it[23]. The creation of a new taxon, the Prochlorophyta, has been recommended to accommodate prokaryotes that contain chlorophylls *a* and *b* and lack phycobilins, to the exclusion of the cyanobacteria[24]. Such a taxon has been previously criticized on the grounds that pigment composition does not constitute sufficient evidence for the creation of a new taxon[25]. This report, that the ability to synthesize chlorophyll *b* occurs sporadically in separate lineages, shows that such a taxon would not constitute a natural grouping. It would be a 'polyphyletic' group, one composed of selected members of independent lineages.

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psbA genes indicate common ancestry of prochlorophytes and chloroplasts

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It has long been suspected that chloroplasts evolved after an endosymbiotic event involving a photosynthetic prokaryote, presumably a cyanobacterium, and a eukaryotic organism. Recent studies have provided strong evidence about the cyanobacterial nature of chloroplasts[1]. Since the discovery of prochlorophytes[2], oxygen-evolving photosynthetic prokaryotes containing chlorophyll *a* and chlorophyll *b* and lacking phycobiliproteins, there has been speculation that these represent evolutionary intermediates between cyanobacteria and chloroplasts[3]. *Prochloron* sp., the first described prochlorophyte, proved difficult to work with because it is an obligate symbiont of marine ascidians[3]. *Prochlorothrix hollandica*, a recently isolated, freshwater filamentous prochlorophyte, is easily maintained in the laboratory[3,4]. Overall pigment composition and thylakoid membrane structure of *P. hollandica* suggest it has intermediate characteristics between cyanobacteria and the chloroplasts of higher plants[3]. The *P. hollandica psbA* genes, which encode the photosystem II thylakoid protein D1, were cloned and sequenced and the sequences compared to those reported for cyanobacteria, a green alga, a liverwort, and several higher plants. The two *psbA* genes present in *P. hollandica* encode an identical amino-acid sequence. As in all chloroplast *psbA* genes, there is a seven amino-acid gap near the C terminus of the derived protein relative to the protein predicted by cyanobacterial genes[5], suggesting that *P. hollandica* is part of the lineage that led to chloroplasts after a divergence from cyanobacteria. This hypothesis is also supported by phylogenetic analysis of derived D1 amino-acid sequences from *psbA* genes of thirteen taxa on the basis of parsimony.

Heterologous *psbA* gene probes from *Synechococcus* sp. strain PCC 7942 (ref. 6) were used to identify regions of sequence similarity in *P. hollandica*. These probes hybridized to two fragments when DNA was digested with either *Eco*RI (6.4 and 5.35 kilobases kb) or *Bam*HI (3.0 and 2.9 kb). Both *Eco*RI fragments were cloned and nucleotide sequence analysis revealed that each clone contained an entire *psbA* open reading frame (Fig. 1). The genes were named such that *psbAI* was sequenced from the 6.4 kb *Eco*RI fragment (which includes the 3.0 kb *Bam*HI fragment) and *psbAII* was sequenced from the 5.35 kb *Eco*RI fragment (which includes the 2.9 kb *Bam*HI fragment). Only two of the 1,059 base pairs (bp) in the open reading frame differed between the nucleotide sequences of the two genes. These differences represent silent transition mutations which did not alter the predicted polypeptide sequences encoded by these genes even though one, nucleotide 1,027, occurred in the first base position of a codon. The number of genes present and the nature of the predicted polypeptide is in contrast to the situation in both cyanobacterial and chloroplast genomes. Al

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Fig. 1 Nucleotide and derived amino-acid sequences of the two *psbA* genes from *P. hollandica*. The nucleotide sequences upstream and downstream of the 1,059 bp open reading frame (ORF) are shown for *psbAI* and *psbAII*. Regions of sequence identity in the upstream and downstream regions of *psbAI* and *psbAII* are underscored. The nucleotide sequence of the *psbAII* ORF is shown in its entirety. Differences in the *psbAI* sequence are denoted above the *psbAII* sequence at positions 282 and 1,027. The single predicted amino-acid sequence for the protein encoded by both *psbAI* and *psbAII* is listed below each corresponding line of nucleotide sequence.

Methods. DNA was digested with *Eco*RI, ligated to *Eco*RI-cleaved λGT-10 cloning vector, and packaged *in vitro*. The library was plated on *Escherichia coli* host strain ER1458 (mcr⁻) and the resulting plaques were transferred to DuPont colony/plaque screen and probed with nick-translated[14] *psbA*-containing fragments from *Synechococcus* sp. PCC 7942. Positively identified plaques were picked, the phage amplified, and DNA isolated[14]. Cloned DNA was subcloned into plasmids for sequencing using a Sequenase dideoxynucleotide kit (United States Biochemical Corporation) and reactions were separated on a 7 M urea, 6% polyacrylamide gel[14]. Nucleotide sequences of different regions of both *psbAI* and *psbAII* were determined by a combination of subcloning restriction fragments, making nested deletions with exonuclease III, and preparing synthetic oligonucleotides from known regions of the genes to use a primers. Sequencing reactions were completed for each gene from overlapping clones to account for all bases on both DNA strands.

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    -80
I   CTATAGTTGG GTCACGTTCA AAGACGGTTA TCCGCACCGA ACGGAAAATT CATACTCTGT ACCTTGCAAT CATAAATACC
II  AAAGTTCACT ATACCGGTAC TATGAGTTCC AACGGTTCAA AAAAACCCGC CTGTACATAG AAAACGAAAT CATAAATACC
                                                                                        66
II  ATG ACA ACC GCA CTT CGC CAG CGT GAA AGC GCT AAT GCG TGG GAA CAG TTT TGC CAG TGG ATC GCC
     M   T   T   A   L   R   Q   R   E   S   A   N   A   W   E   Q   F   C   Q   W   I   A
                                                                                       132
II  AGC ACC GAG AAC CGC CTG TAT GTG GGT TGG TTC GGC GTG ATC ATG ATC CCC ACC CTG CTG ACT GCC
     S   T   E   N   R   L   Y   V   G   W   F   G   V   I   M   I   P   T   L   L   T   A
                                                                                       198
II  ACC ATC TGC TTC ATC ATC GCC TTC ATC GCT GCT CCC CCC GTG GAC ATC GAC GGT ATC CGT GAG CCT
     T   I   C   F   I   I   A   F   I   A   A   P   P   V   D   I   D   G   I   R   E   P
                                                                                       264
II  GTT GCT GGT TCC TTG ATG TAC GGC AAC AAC ATC ATC TCC GGT GCT GTG GTT CCC TCC TCC AAC GCG
     V   A   G   S   L   M   Y   G   N   N   I   I   S   G   A   V   V   P   S   S   N   A
                                                                                       330
I                        T
II  ATC GGT CTG CAC TTC TAC CCC ATT TGG GAA GCA GCC AGC ATG GAC GAG TGG CTC TAC AAC GGT GGT
     I   G   L   H   F   Y   P   I   W   E   A   A   S   M   D   E   W   L   Y   N   G   G
                                                                                       396
II  CCT TAC CAG TTG GTG GTG TTC CAC TTC CTG ATC GGC ATC TTC TGC TAC ATG GGT CGT GAG TGG GAA
     P   Y   Q   L   V   V   F   H   F   L   I   G   I   F   C   Y   M   G   R   E   W   E
                                                                                       462
II  CTC TCC TAC CGC CTT GGG ATG CGT CCC TGG ATC TGT GTC GCT TAC TCC GCT CCT GTG GCC GCT GCG
     L   S   Y   R   L   G   M   R   P   W   I   C   V   A   Y   S   A   P   V   A   A   A
                                                                                       528
II  ACC GCT GTG TTC TTG ATC TAC CCC TTG GGC CAA GGT TCC TTC TCT GAT GGA ATG CCC CTG GGT ATC
     T   A   V   F   L   I   Y   P   L   G   Q   G   S   F   S   D   G   M   P   L   G   I
                                                                                       594
II  TCT GGT ACC TTC AAC TTC ATG TTG GTC TTC CAG GCT GAG CAC AAC ATC CTG ATG CAC CCC TTC CAC
     S   G   T   F   N   F   M   L   V   F   Q   A   E   H   N   I   L   M   H   P   F   H
                                                                                       660
II  ATG CTC GGT GTG GCT GGT GTC TTC GGT GGT TCC TTG TTC TCC GCC ATG CAC GGT TCT TTG GTC ACC
     M   L   G   V   A   G   V   F   G   G   S   L   F   S   A   M   H   G   S   L   V   T
                                                                                       726
II  TCC TCC TTG GTT CGT GAA ACC TCT GAG AAC GAG TCC CAG AAC TAC GGC TAC AAG TTC GGT CAA GAA
     S   S   L   V   R   E   T   S   E   N   E   S   Q   N   Y   G   Y   K   F   G   Q   E
                                                                                       792
II  GAA GAG ACC TAC AAC ATC GTG GCT GCC CAC GGT TAC TTC GGT CGC CTG ATC TTC CAA TAT GCT TCT
     E   E   T   Y   N   I   V   A   A   H   G   Y   F   G   R   L   I   F   Q   Y   A   S
                                                                                       858
II  TTC AAC AAC AGC CGT GCC CTG CAC TTC TTC CTC GCT GCT TGG CCC GTT GTC GGC ATC TGG TTT ACC
     F   N   N   S   R   A   L   H   F   F   L   A   A   W   P   V   V   G   I   W   F   T
                                                                                       924
II  TCT CTG GGT ATC TCC ACC ATG GCC TTC AAC CTC AAC GGT TTC AAC TTC AAC CAG TCC GTG ATG GAC
     S   L   G   I   S   T   M   A   F   N   L   N   G   F   N   F   N   Q   S   V   M   D
                                                                                       990
II  AGC CAA GGT CGC GTT ATC AGC ACC TGG GCC GAC ATC CTC AAC CGT GCC AAC CTC GGT TTT GAA GTC
     S   Q   G   R   V   I   S   T   W   A   D   I   L   N   R   A   N   L   G   F   E   V
                                                    T                                  1056
I   ATG CAC GAG CGC AAC GCT CAC AAC TTC CCC CTT GAC CTG GCT GCT GTC AAA GCT CCT TCC ATC ATC
II   M   H   E   R   N   A   H   N   F   P   L   D   L   A   A   V   K   A   P   S   I   I
                                                                                      1132
I   GGT TAA GTTGAGTTGC TCTACTCATC TCCTAGCCTA GGCTAGACCT AAAGCGTCTC TTGGGGGGGC GTTTTTGCGT
II  GGT TAA GTTGAGTTGC ACTACTCATC TAATAGCCTA GGCTAGACCT AAAGCGTCCT CCTTCGGGGG GGCGCTTTTT
     G   *
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cyanobacteria that have been examined have three or more copies of *psbA* in their genomes which encode two different polypeptides[5,6] whereas the small circular chromosome of most chloroplasts contains a single copy of *psbA*[7].

The predicted D1 polypeptide of cyanobacteria contains 360 amino acids compared to 353 amino acids in *P. hollandica* and higher plants (352 amino acids in *Chlamydomonas*). Figure 2 shows a comparison of the C-terminal region of the D1 protein predicted from *psbA* genes from various cyanobacteria, eukaryotic species, and *P. hollandica*. Plastid and *P. hollandica* polypeptide sequences all share a seven amino-acid gap relative to the cyanobacterial sequences at a position six amino acids from the end of the polypeptide. The presence of well-documented insertion/deletion events in bacterial genomes has been implicated as a more reliable indicator of phylogenetic relationships than nucleotide or amino-acid sequences[8]: the definitive gap among the *psbA* genes of specific lineages suggests a more recent common ancestor among green plant chloroplasts and *P. hollandica* than among chloroplasts and cyanobacteria.

Cyanophora paradoxa (a photosynthetic protozoan) has been implicated as a descendant of the progenitor to green chloroplast-bearing organisms because of the presence of phycobiliproteins, rather than chlorophyll *b*, in the chloroplast-like cyanelle[9]. Comparisons of 16S rRNA sequences, however, show that *C. paradoxa* is not directly related to plastids and probably represents an independent endosymbiotic event[1]. They also suggest that a cyanobacterium, possibly similar to the endosymbiont of *C. paradoxa*, was probably the progenitor of chloroplasts[1]; however, a prochlorophyte was not included in that study. The *psbA* gene of *C. paradoxa* has recently been sequenced (W. Löffelhardt, personal communication). The seven amino acids characteristic of cyanobacterial sequences, but lacking in chloroplast-containing organisms, are present in the sequence of *C. paradoxa*. This strengthens the correlation between phycobiliprotein-containing organisms having a D1 protein of 360 amino-acid residues and chlorophyll *b*-containing organisms lacking the seven amino-acid domain at the C terminus of the protein. That *P. hollandica* also lacks this domain is further evidence that prochlorophytes, *P. hollandica* in particular, are members of the same lineage as the endosymbiont chloroplast progenitor which diverged from cyanobacteria. If prochlorophytes are not of this lineage, both chlorophyll *b* and *psbA* sequences shortened at the same position were derived independently in the two lineages.

As a further test to determine the relationships of *P. hollandica* to cyanobacteria and green chloroplast-containing organisms, the predicted polypeptide sequences of *psbA* genes from several species of vascular plants, a non-vascular plant, a green alga, four cyanobacteria, and *P. hollandica* were compared using PAUP, a computer program designed to determine phylogenetic

Anabaena 7120	(1)	**LDLAA**GEVAPVALTAPAING*
	(2)	**LDLAA**GEVAPVAISAPAING*
Fremyella diplosiphon		**LDLAA**GEVAPVALTAPAING*
Synechococcus 7942	(1)	**LDLAA**GEATPVALTAPSIHG*
	(2)	**LDLAA**GEATPVALTAPAING*
Synechocystis 6803		**LDLA**SGDAQMVALNAPAIEG*
Chlamydomonas		**LDLA**STN-------SSSNN-*
Marchantia polymorpha		**LDLAA**VE-------APAVNG*
Spinach		**LDLAA**IE-------APSTNG*
Soybean		**LDLAA**ID-------APSING*
Alfalfa		**LDLAA**VE-------APSING*
Prochlorothrix hollandica		**LDLAA**VK-------APSIIG*

Fig. 2 Comparison of amino-acid sequences at the C terminus of the D1 polypeptide from different genera: *Anabaena*[5], *Fremyella diplosiphon*[15], *Synechococcus*[6], *Synechocystis*[16], *Chlamydomonas*[17], *Marchantia polymorpha*[18], spinach[19], soybean[20], alfalfa[21], *Prochlorothrix hollandica*. The C-terminal amino acids from residue 341 to the termination codon (*) are shown for each sequence, and those conserved among all sequences analysed are in bold type. Numerals following the genera *Anabaena*, *Synechococcus*, and *Synechocystis* refer to strain designations of the Pasteur Culture Collection. The *psbA* gene families of *Anabaena* sp. strain 7120 and *Synechococcus* sp. strain 7942 encode two slightly different D1 proteins; in each case the product of *psbA1* is designated by (1) and the product of the other members of the gene family is designated (2). Other plants with sequences identical to spinach in this region are *Amaranthus hybridus*[22], *Nicotiana debneyi*[19], *N. tabacum*[23], *Petunia hybrida*[24], and *Solanum nigrum*[25].

relationships based on parsimony[10]. Sequences of all taxa were aligned and were analysed by treating the gap region in four different ways: (1) excluding amino acids at positions 348–354 of cyanobacterial sequences from the analysis to compare the 353 remaining amino acids; (2) treating the seven amino-acid positions in the gap region as a single binary character (that is, presence or absence of this domain); (3) weighting each position of the gap region by 0.14 so the entire region is equal to a single character change with no loss of information at each position; (4) considering each position of the gap region as a separate and unique character. When amino acids of the gap region were completely excluded from the analysis (treatment 1), 30 equally parsimonious trees were produced, 15 of which were similar to that shown in Fig. 3 and 15 that associated *P. hollandica* in a lineage with the two *Synechococcus* sequences. The additional trees within each of these are accounted for by polychotomies and minor differences in topology of branches leading to higher plants. When the gap region was included in the analysis in any way (treatments 2, 3 or 4), tree topology was the same as in Fig. 3 with the major difference among the four treatments being the length of the branch (labelled *a*) leading to *P. hollandica* and chloroplast lineages: *a* was shortest in treatment 1 and longest in treatment 4 (data not shown). The length of each branch on the tree reflects the number of character state differences unique to that branch and the amount of divergence from a most recent common ancestor. The phylogenetic trees produced thus corroborate the phylogeny inferred from the gap distribution which places *P. hollandica* closer than cyanobacteria to green plant chloroplasts.

The predicted rate of divergence among highly conserved genes in bacteria has been estimated to be approximately 1% per 50 million years (Myr)[11,12]. The percentage similarity among *P. hollandica psbA* nucleotide sequences and those of *Anabaena* or *Synechococcus* range from 81.6% to 83.6% for the six genes examined, which would place the time of divergence between the cyanobacterial and prochlorophyte lines at about 900 Myr ago. This figure is within the range (800–2,000 Myr ago) previously predicted for the time of divergence between plastids and cyanobacteria based on 16S rRNA comparisons[11,12].

With the discovery of a third member of the *Prochlorophyta* which occupies deep marine euphotic zones[13], studies on this interesting group should continue to contribute to our understanding of the phylogeny of chloroplasts. Because *P. hollandica* is easily grown in culture[3,4] and represents an organism that

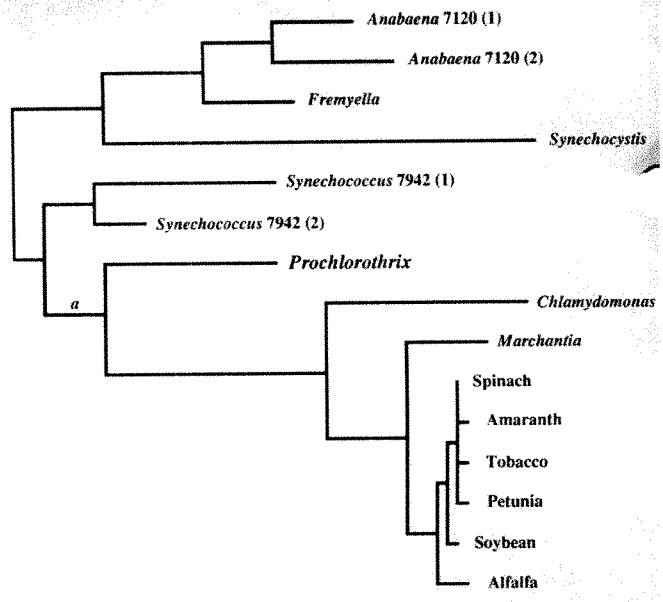


Fig. 3 Phylogenetic tree based on parsimony[10] among amino-acid sequences derived from *psbA* gene sequences of thirteen species. Branch length reflects the number of character state differences unique to that branch and the amount of divergence from a most recent common ancestor, but is not meant to depict evolutionary time. The branch labelled *a* was found to be variable in length among the four different analyses (see text). Gene designations for *Anabaena* sp. strain PCC 7120 and *Synechococcus* sp. strain PCC 7942 are as in Fig. 2. Sequences identical to that of spinach and not shown are *Nicotiana debneyi*[19] and *Solanum nigrum*[25]. Data for sequences were obtained from the same data set as in Fig. 2.

Methods. 118 positions along the amino-acid sequences were variable for at least one of the 15 sequences analysed. Amino acids were converted to a numeric character for each of the variable positions. The following numbers of equally parsimonious trees were generated for each of the four treatments used to compare sequences: (1) 30 trees, (2) 15 trees, (3) 15 trees, and (4) 15 trees. The order of branching was the same in all trees for treatments 2–4 and in half of the trees generated using treatment 1 (see text), with some minor differences in the branching order of higher plants. Amino acids 348–354 at the C terminus of cyanobacterial sequences were excluded from the analysis which produced this tree. All data were considered unordered and the tree was produced rooted at the midpoint of the greatest patristic distance using the branch-and-bound option of PAUP[10].

seems to be more chloroplast-like than cyanobacteria, *P. hollandica* may serve as a useful model organism for the study of oxygenic photosynthetic mechanisms.

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Bacterial growth blocked by a synthetic peptide based on the structure of a human proteinase inhibitor

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Cysteine proteinases are important not only in the intracellular catabolism of peptides and proteins[1] and in the processing of prohormones and proenzymes[2,3], but also in the penetration of normal human tissue by malignant cells[4] and possibly microorganisms[5], including viruses. Cystatin C is a human cysteine proteinase inhibitor present in extracellular fluids[6]. We have synthesized peptide derivatives mimicking the proposed proteinase-binding centre of cystatin C[7] and find that they irreversibly inhibit cysteine proteinases. Several bacteria produce proteinases, so we tested a tripeptide derivative (Z-LVG-CHN_2) for *in vitro* anti-bacterial activity against a large number of bacterial strains belonging to thirteen different species. It was found to inhibit specifically the growth of all strains of group A streptococci. The susceptibility of these human pathogens to the peptide was compared with that to well-established anti-streptococcal antibiotics such as tetracycline and bacitracin. Moreover, the peptide was active *in vivo* against group A streptococci: mice injected with lethal doses of these bacteria were cured by a single injection of Z-LVG-CHN_2. The cysteine proteinase produced by group A streptococci was isolated and found to be inhibited by Z-LVG-CHN_2; moreover, excess proteinase relieved the growth inhibition caused by the peptide derivative, suggesting that the antibacterial activity of Z-LVG-CHN_2 is due to inhibition of this cysteine proteinase. This strategy of blocking proteinases with peptide derivatives that mimic naturally occurring inhibitors could be useful in the construction of new agents against other microorganisms, including viruses.

Of the peptide derivatives we synthesized to mimic the inhibitory reactive site of cystatin C (ref. 7), *N*-benzyloxycarbonyl-leucyl-valyl-glycine diazomethyl ketone (Z-LVG-CHN_2) most effectively inhibited papain, a model cysteine proteinase (association rate constant, $k'_{+2} = 600{,}000\ mol^{-1}\ s^{-1}$). The peptidyl portion of Z-LVG-CHN_2 was acting as a supersubstrate for the inhibited cysteine proteinase; the diazomethyl ketone group established a covalent link with the cysteine residue in the active site of cysteine proteinases[9], thereby making Z-LVG-CHN_2 an irreversible inhibitor.

Many microorganisms produce proteinases, so we investigated the effect of Z-LVG-CHN_2 on bacterial growth. Paper discs were impregnated with the synthetic inhibitor and applied to plates streaked with different bacteria (see legend to Fig. 1). Growth of all group A streptococcal strains tested was inhibited to a degree comparable with that shown by some well established antibiotics (Fig. 1); all other strains were unaffected by Z-LVG-CHN_2. We then analysed the anti-streptococcal activity of Z-LVG-CHN_2 in infected mice using a strain of group A streptococcus virulent in mice (strain 40/58). Ten outbred NMRI mice (ALAB, Sweden) were injected intraperitoneally (i.p.) with 10^6 streptococci in Todd–Hewitt broth. Thirty minutes later we injected (i.p.) five animals with 0.2 mg Z-LVG-CHN_2 in 0.5 ml 0.15 M NaCl in 1% dimethyl sulphoxide and five with 0.5 ml 0.15 M NaCl in 1% DMSO without Z-LVH-CHN_2. All mice in the control group died within 24 h, whereas among those treated with Z-LVG-CHN_2, one mouse died after three days and the

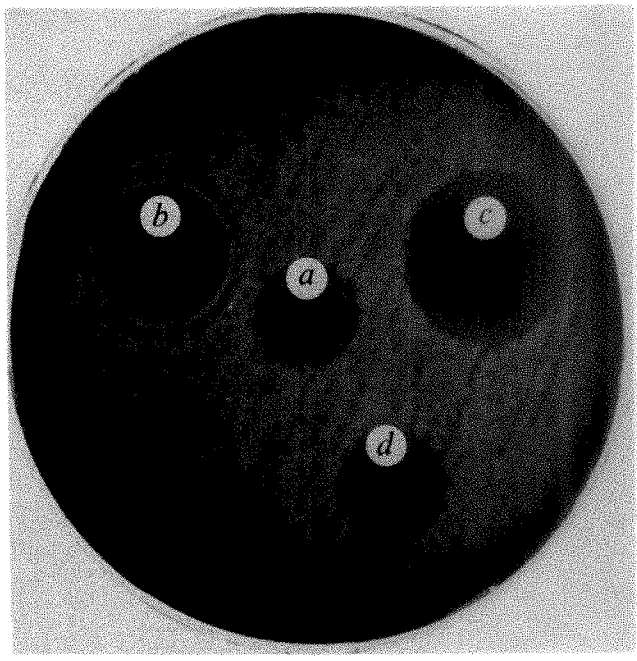


Fig. 1 Anti-bacterial activity of Z-LVG-CHN_2 in disc diffusion analysis.
Methods. Streptococci (strain 40/58 from the WHO Collaborating Center for Reference and Research on Streptococci, Institute of Hygiene and Epidemiology, Czechoslovakia) were plated on 0.8% purified agar in Todd–Hewitt broth containing 4% sheep's blood, and antibiotic-sensitivity discs were applied to the plate. In *a*, a non-impregnated disc had been prepared to contain 24 μg Z-LVG-CHN_2 by impregnating a sterile filter paper disc with 60 μl Z-LVG-CHN_2 (0.4 mg ml^{-1}) in 1% dimethyl sulphoxide in water. Disc *b* contained 30 μg doxycycline; disc *c*, 0.1 μg benzylpenicillin; disc *d*, 0.2 IU of bacitracin. All discs were from AB Biodisc, Sweden. Z-LVG-CHN_2 discs (24 μg per disc) were also used to screen a total of 190 strains of human pathogenic bacteria. All strains tested were from routine clinical specimens (Clinical Microbiology Laboratory, University Hospital in Lund). Species and number of strains were: *Staphylococcus aureus*, 12; *Staphylococcus epidermidis*, 14; group A streptococci, 28; group B streptococci, 14; group C streptococci, 19; group G streptococci, 11; *Streptococcus pneumoniae*, 8; *Streptococcus faecalis*, 10; *Branhamella catarrhalis*, 9; *Haemophilus influenzae*, 17; *Escherichia coli*, 22; *Klebsiella pneumoniae*, 15 and *Pseudomonas aeruginosa*, 11. All group A streptococcal strains showed a zone similar to *a* in the figure, with no bacterial growth surrounding the disc, whereas the other strains grew up to the edge of the Z-LVG-CHN_2-containing disc. In control experiments eight of the group A streptococcal strains were also tested against discs impregnated with 60 μl 1% dimethylsulphoxide or with 24 μg commercial (Sigma) cysteine proteinase inhibitor L-3-carboxy-*trans*-2,3-epoxypropionyl-leucylamido-(4-guanidino) butane (E-64). No inhibitory activity was shown by either the dimethylsulphoxide solution or by E-64.

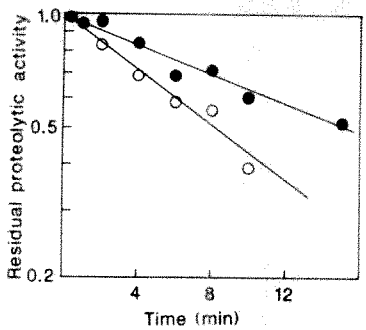


Fig. 2 Time-dependent inactivation of isolated streptococcal proteinase by 10 μM (●) or 20 μM (○) Z-LVG-CHN_2.

Methods. The streptococcal proteinase was isolated from growth medium of the streptococcal group A strain 40/58 from the Prague collection, as previously described[8]. The isolated enzyme was activated by preincubation at 25 °C during 1 h in 0.2 M sodium acetate/10 μM EDTA/50 μM dithiothreitol, *p*H 5.5. The activated enzyme (7.5 μg ml^{-1}) and Z-LVG-CHN_2 (from a stock solution in 1% dimethylsulphoxide) were mixed in the activation buffer at zero time. Samples were taken out at timed intervals and immediately added to assay tubes containing the chromogenic substrate Z-Lys-ONp (*N*-α-carboxybenzoyl-L-lysine-paranitrophenyl ester HCl (Bachem) at a final concentration of 0.2 mM. After 20 min at 25 °C, the reaction was stopped by addition of buffered chloroacetate, and the absorbance was read at 340 nm. A time-dependent inactivation of the enzyme was found. The residual proteolytic activity in the samples as a function of time gave a linear semilogarithmic plot typical of an irreversible second-order reaction. The rate of inactivation ($t_{0.5}$) at different Z-LVG-CHN_2 concentrations (10 μM and 20 μM) was dependent on the concentration of Z-LVG-CHN_2 ([*I*]). The apparent rate constant for association between enzyme and inhibitor, k'_{+2}, was then calculated as $\ln 2/t_{0.5}[I]$ (ref. 10).

remainder were still alive after two months. In a repeat experiment, all five control animals died within 24 h and the five mice treated with Z-LVG-CHN_2 survived (four weeks observation time). We then investigated the toxicity of Z-LVG-CHN_2: no ill-effects were seen in four mice each injected i.p. with 2 mg in 5 ml 0.15 M NaCl in 1% dimethylsulphoxide (two months observation time). So Z-LVG-CHN_2 is atoxic even at doses tenfold higher than those necessary to cure lethal streptococcal infections in mice.

Although Z-LVG-CHN_2 could inhibit streptococcal growth both *in vivo* and *in vitro*, the molecular mechanism behind this activity was not clear. There is a cysteine proteinase that is produced by all group A streptococcal strains but which has not been identified in other bacterial species, including other species of streptococci; the enzyme is produced as a zymogen that can be transformed into an active proteinase by reduction[8]. We purified the zymogen to apparent homogeneity from growth medium containing the same group A strain as we used in the animal experiments, when we found that the activated enzyme was irreversibly inhibited by Z-LVG-CHN_2. The association rate constant (k'_{+2}) of the reaction was determined as 102 mol^{-1} s^{-1} (Fig. 2). Moreover, Z-LVG-CHN_2-mediated growth inhibition could be impeded by addition of the activated proteinase. These data indicate that Z-LVG-CHN_2 could be inhibiting growth of group A streptococci by blocking their cysteine proteinase. E-64 is a well-known cysteine proteinase inhibitor[10], whose activity against the isolated streptococcal enzyme is comparable with that of Z-LVG-CHN_2, but E-64 did not inhibit growth of group A streptococci. This might be explained by the relatively greater hydrophobicity of Z-LVG-CHN_2 facilitating penetration of the bacterial cell wall, or by the fact that Z-LVG-CHN_2 is recognized by a permease, whereas E-64 is not. On this basis, proteinase-inhibiting peptide derivatives could be made more useful for antimicrobial therapy if their N-termini were recognizable by permeases that permit efficient intracellular uptake[11].

The biological function of the streptococcal proteinase is n known, but our data indicate that blocking the enzyme inhibi growth of these bacteria both *in vivo* and *in vitro*, which suggest that the proteinase is essential for the multiplication of grou A streptococci. This is supported by the fact that we have s far been unable to isolate any mutants of group A streptococ devoid of the cysteine proteinase, neither have we seen an Z-LVG-CHN_2-resistant mutants of the 40/58 strain (frequenc less than 10^{-6}). It will be interesting to see which of the othe microorganisms that are known to require proteinases for multi plication and/or tissue penetration are affected by inhibitor that imitate supersubstrate sections of naturally occurring pro teinase inhibitors.

We thank Inga-Maria Frick and Ingbritt Gustafsson for tech nical assistance. This work was supported by the Swedis Medical Research Council, the Medical Faculty at the Universit of Lund, the Österlund Foundation, and the Anna-Gret Crafoord Foundation for Rheumatology Research.

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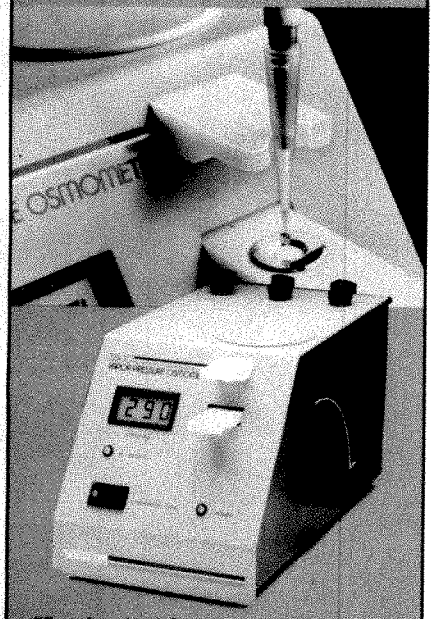
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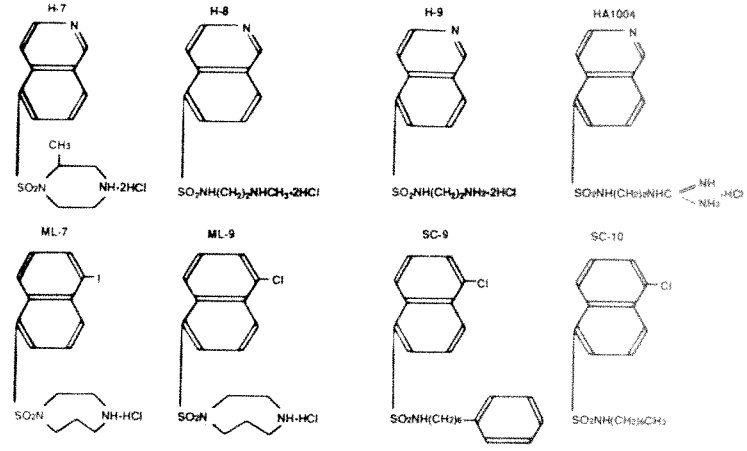
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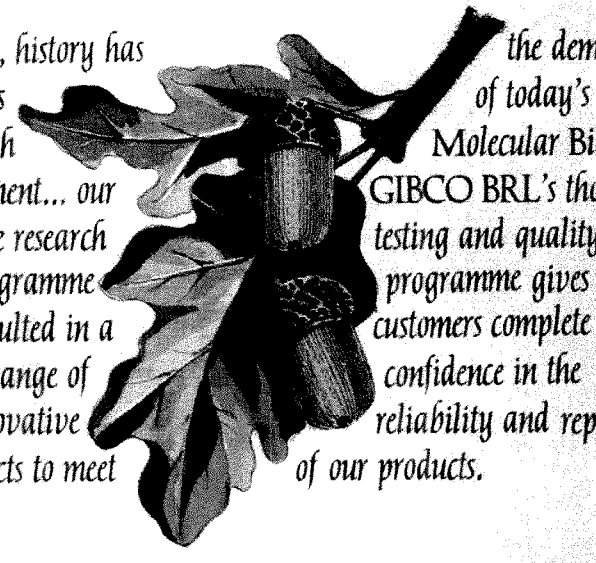
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Cationic liposome-mediated transfection

P.L. Felgner and G.M. Ringold

A lipid named DOTMA has been created that forms unilamellar liposomes which complex with DNA and RNA for the transfection of mammalian cells, including suspension cells and hybridomas.

EFFORTS to develop methods for functionally delivering polynucleotides into living cells have continued steadily over the past several years. Effective methods include the use of calcium phosphate, liposomes, retroviral vectors, reconstituted viral envelopes and electroporation. In addition, there are several procedures which employ polycations such as polylysine, DEAE-dextran and polyornithine[1-3].

Recently, a DNA delivery reagent was developed which consists of polycationic liposomes comprised of a positively charged lipid named DOTMA, for (*N*[1-(2,3 - dioleyloxy)propyl] - *N,N,N* - triethylammonium)[3]. The advantages of this reagent over other methods have been demonstrated for a variety of cell types, and include improvements in convenience, reproducibility, efficiency and efficacy.

The reagent is particularly notable for its ease of use. An aliquot of the aqueous reagent is simply added to the tissue culture experiment together with the DNA or RNA of interest. Although the level of observed expression is heavily dependent upon the cell line and expression vector, in the vast majority of cases, expression levels are at least comparable to — and frequently much greater than — those obtained with either the calcium phosphate or DEAE-dextran methods[3].

Mechanism of action

The postulated mechanism of action for the liposome reagent, based on experimental evidence, is illustrated in Fig. 1. DOTMA forms liposomes in an aqueous environment alone or together with other phospholipids[3,4], and the active reagent consists of sonicated vesicles containing equal parts of DOTMA and dioleoylphosphatidylethanolamine in water. DNA/DOTMA complexes form spontaneously after mixing an aliquot of the reagent with an aqueous solution of DNA[3], trapping nearly all of the polynucleotide in the complex interior.

The ratio of DOTMA to DNA is critical to the reagent's efficacy[3,5,6]. Theoretically, the optimal ratio should be such that the amount of positive charge contributed by the DOTMA liposomes slightly exceeds the number of negative charges on the DNA, so that the complexes have a net positive charge to facilitate their interaction with the negatively charged target cell surface. In practice, however, the optimum quantity of DOTMA is usually found to be roughly half this amount, and it is best to calibrate the concentration for each specific system to be studied.

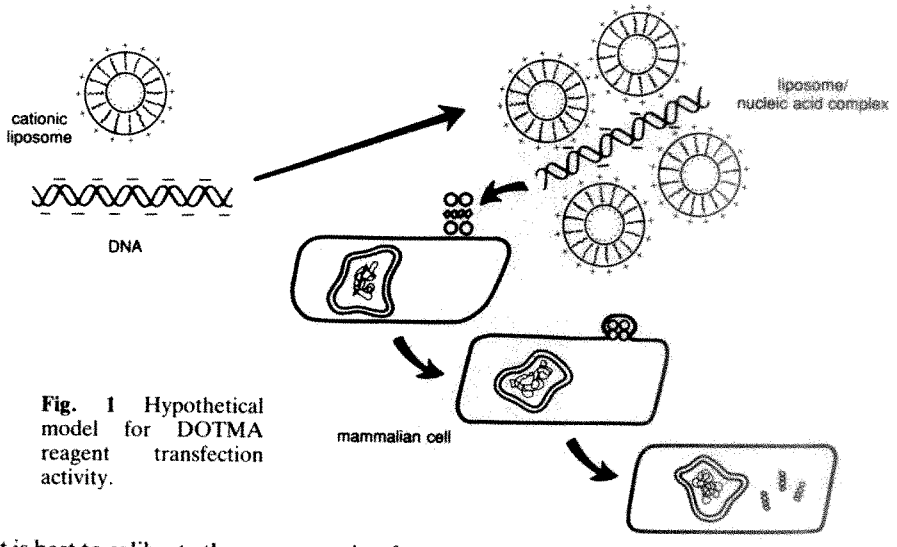


Fig. 1 Hypothetical model for DOTMA reagent transfection activity.

We hypothesize that DNA/DOTMA complexes consist of four liposomes in association with each plasmid. Liposomes that are 250 nm in diameter — the approximate size of the vesicles in our reagent — contain about 2,500 lipid molecules[7]. Half of the molecules in each liposome, or 1,250 molecules, are positively charged DOTMA molecules. Because 5,000 negative charges are present in a standard 2,500-base-pair plasmid, approximately four liposomes are necessary to neutralize the negative charges of the plasmid DNA. The average diameter of the complexes is only slightly larger than that of the original liposomes as measured by laser light scattering (unpublished data), indicating that they contain a very small number of plasmids (perhaps only one) per complex. Complexes containing a larger number of plasmids would show a substantial, measurable size increase.

Liposomes comprised of DOTMA and dioleoylphosphatidylethanolamine fuse with negatively charged lipid membranes and with cell surfaces[4]. Micrographs of cells exposed to fluorescent DNA/liposome reagent complexes which contain rhodamine-labelled lipid also indicate cell-surface fusion[3]. Comparable fusion results obtained with a positively charged lipid analogue which is structurally very similar to DOTMA have recently been published[8]. Liposomes comprised of stearylamine or dioctaldecyl-dimethylammonium bromide were inactive in transfection assays (unpublished data).

Different subpopulations of cells and cell types seem to take up DNA/DOTMA complexes with equal affinity. Following transfection with DNA/DOTMA complexes containing a fluorescent lipid analogue, essentially 100 per cent of cells become fluorescent[3]. Furthermore, cells exposed to radioactively labelled complexes adsorb 20-80 per cent of the added polynucleotide[6]. In a typical experiment in which 2 μg of a 2,500-base pair plasmid are exposed to one million cells (with approximately 10^6 plasmids per cell) about 500,000 plasmids would be expected to be taken up per cell. But at the end of a two-day transfection experiment with mouse L- cell fibroblasts, only 300 intact psV2-CAT plasmids per cell were recovered in the nuclear fraction (unpublished data), indicating that less than one per cent of the adsorbed plasmids were reaching the nucleus intact. In a parallel experiment using the calcium phosphate method, however, less than 10 plasmid copies per cell were found in the nucleus.

In histochemical staining experiments on cells transfected with β-galactosidase expression vectors, up to 25 per cent of the cells were positive. It is not known whether the remaining 75 per cent of the cells were truly negative, or whether they were expressing activity below the sensitivity of the staining procedure: experiments with double-stranded RNA clearly show that more than 99 per cent of the cells are transfected[5]. Stable transformation efficiencies of as high as 10 per cent have been obtained, although this varies depending on vector and cell type.

The transfection step is performed in serum-free media to overcome the effects of an inhibitory component present in serum. The duration of this incubation

period ranges from 2 to 24 hours. Because purified sulphated proteoglycans are potent inhibitors of DOTMA-mediated transfection (unpublished results), a similar polyvalent negatively charged serum component is presumably responsible for this effect.

The efficiency of the method is essentially unaffected by variances in *p*H between 6.0 and 8.0[3]. Where a *p*H difference of as little as 0.1 in a calcium phosphate transfection experiment can influence the result, it has no effect on the DOTMA reagent[1]. Precise mixing protocols, careful attention to the level of DNA, and the presence of low levels of contaminants are also less important to the outcome of DOTMA-mediated transfection experiments. In addition, large DNA molecules such as baculovirus DNA (130 kilobase pairs) have been successfully transfected into tissue culture cells using the technique (J. Barnett, personal communication).

Applications

Detailed quantitative data comparing the DOTMA reagent with other optimized transfection methods is available for only a few selected systems[3], but some general statements regarding relative efficacy are possible. As with all transfection methods, the transient expression levels and transformation efficiencies obtained are strongly dependent upon the particular expression vector and the cell line to be tested. In a number of cell lines, 10- to 100-fold improvements over other methods have been demonstrated[3]. The reagent has also been used to transfect cells unresponsive to other methods (such as PC12 cells), suspension cells and hybridomas. The DOTMA liposome reagent has been used successfully to transfect, both stably and transiently, over 50 cell lines and primary cell cultures.

Relatively crude preparations of plasmid DNA, such as those made in minipreps, can be efficiently introduced into cells using the reagent, allowing large numbers of different DNAs to be screened. For example, the products of site-directed mutagenesis can be conveniently transfected without time-consuming purification protocols.

Particularly exciting is the recent finding that the reagent is broadly applicable for the delivery and expression of messenger RNA in a wide variety of both adherent and suspension culture cell lines[5]. This has opened up a new experimental approach to the characterization of mRNA structure and function, areas that were not possible to study using more conventional transfection procedures. For example, the method has shown that the expression efficiency of mRNA synthesized *in vitro* is strongly dependent upon the presence of specific untranslated elements in the RNA construct[6]. The reagent has also been used successfully to deliver Sinbus virion RNA or *in vitro*-transcribed infectious RNA, resulting in active virus particles (S. Schlesinger, personal communication).

Despite the advantages offered by the liposome reagent, other transfection protocols may be more effective for some applications. A notable example is the optimized calcium phosphate procedure of Okayama[1], which gives excellent results in some cell lines. Similarly, for many suspension cultures, electroporation continues to be the only effective method for introducing nucleic acids. Despite these observations, the DOTMA-mediated transfection procedure stands out as one that can be broadly, successfully and conveniently applied to a a very wide variety of different cell types for both stable and transient expression, and which can be applied to other polynucleotides, including both double-stranded[5] and single-stranded messenger RNA[6]. Future studies with reagents of this type should reveal even more efficacious delivery systems for the introduction of small oligonucleotides, very large DNA fragments or even proteins into intact cells.

The DOTMA reagent is now commercially available from Bethesda Research Laboratories (Bethesda, Maryland USA) under the name Lipofectin. Still other cationic liposome delivery systems may eventually prove applicable to the delivery of polynucleotides *in vivo* for therapeutic applications. □

Philip L. Felgner is at Vical, Inc., 9373 Town Centre Drive, Suite 100, San Diego, California 92121, USA. Gordon M. Ringold is at Syntex Research, 3401 Hillview Avenue, Palo Alto, California 94304, USA. *For a table listing the cell lines which have successfully been transfected using Lipofectin, fill in reader service number 100.*

1. Chen, C.A. & Okayama, H. *Biotechniques* **6**, 632-63 (1988).
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Cell-to-cell communication

Products for examining the intricacies of cell function will be on hand next week when the American Society for Cell Biology and the American Society for Biochemistry and Molecular Biology hold their joint meeting in San Francisco, California. Below are several examples.

PROMEGA now offers confidential **cDNA and genomic library construction** services *(Reader Service No. 101)*. The company will take polyadenylated RNA or genomic DNA, and construct a library using their line of vectors, including those prepared from lambda gt10, lambda gt11 and lambda gt11 *Sfi- Not*. These vectors allow cloning strategies such as directional cloning, subtraction library construction, and expression of cloned inserts as polypeptides fused with β- galactosidase. Each lambda library is packaged *in vitro* using Promega's Packagene extract, which the company says is prepared from a single strain devoid of *Eco* K activity to avoid destroying inserts with that restriction site. Promega will also isolate RNA from tissue, screen libraries, prepare plasmids and phage DNA, and produce antisera on a contract basis.

Benchtop power

Flow has a Cell Raiser of a **benchtop bioreactor** that prevents sleepless nights of worry and tedious trips to the lab to check growth parameters *(Reader Service No. 102)*. Flow's bioreactor of that name comes complete with its own data acquisition system, so researchers can keep an eye on fermentation data from home or a separate office using a modem. The Cell Raiser has built-in controls for *p*H, dissolved oxygen, agitation and temperature. Using its diagnostic software, the bioreactor's controller continuously tests its control loops, including tests for sensor operation and control elements. If any control value deviates from parameters set by the user, it sets off visual and



Flow's Cell Raiser bioreactor.

udible alarms. The computer can also be et to call users remotely via a modem to eliver alarm information. What's better, sers can alter parameter settings through he modem to fix minor problems.

Jouan has a **midrange benchtop centrifuge** that can be used to spin microtubes nd bottles — without changing the rotor *(Reader Service No. 103)*. Jouan's model 2312 centrifuge comes with rotors equipped with plastic insert modules onfigured for labware ranging from 0.5 nl tubes to 250 ml bottles. The modules



Jouan's benchtop centrifuge comes with modular plastic tube inserts.

allow several types of samples to be centrifuged in one run, in different wells of the same rotor. The microtube inserts can also be used as a tube rack, so that experiments can be whisked from the benchtop directly to the centrifuge. The centrifuge's horizontal T4 rotor achieves up to 3,600 r.p.m., generating 2,550 *g*. The fixed angle rotor generates up to 6,300 *g*. The C312 centrifuge comes in both a non- refrigerated version and a refrigerated version which has a temperature range of 0–30 °C.

Yamato has incorporated the innovative technology of molecular sieving into a **freeze dryer** that fits onto the lab bench *(Reader Service No. 104)*. Yamato has altogether eliminated bulky cooling mechanisms in its model DC41 freeze dryer: the unit silently "cages" sublimating water vapour molecules without dry

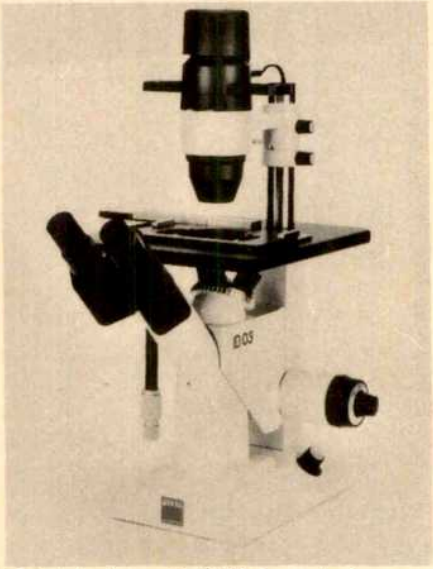


Yamato's bath-free freeze dryer.

ice, chilled alcohol baths or mechanical refrigeration. Heating the sieves releases the accumulated water and regenerates them for reuse. The freeze dryer has five ports, and can process 200 ml of sample at one time. Its instrument panel has a timer with a buzzer to signal the conclusion of the sublimination, and either a thermocouple vacuum gauge or a Bourdon Tube for measuring pressure in centimetres of mercury. The company says its freeze dryer is particularly useful for the preservation of temperature-sensitive biologicals.

The small world

Zeiss has rounded out its line of **inverted microscopes** with its low-priced model ID03 *(Reader Service No. 105)*. The inverted microscope features a new 6 V, 20 W illuminator with a diffusing disk that Zeiss says assures even illumination in brightfield techniques. It contains an aperture iris diaphragm and a three-position slider that allows it to be switched instantly from brightfield to either of two phase-contrast techniques. The new illuminator in the model ID03 has a working



Zeiss's basic model IDO3 inverted microscope comes with a host of features.

distance of 70 mm at 0.3 numerical aperture, and its housing has a flip-top for replacing the bulb.

Olympus's new SZH model **zoom stereomicroscopes** have a remote control facility for hands-free operation *(Reader Service No. 106)*. Focusing is controlled using a foot pedal — a feature that Olympus says is particularly useful when dissecting using micromanipulation tools. Firm foot pressure is used to make rapid movements, and fine focusing is controlled by pressing gently. An anti-crash sensor prevents damage to the specimen or microscope. Olympus SZH stereomicroscopes have an 8.5:1 zoom ratio and a potential magnification range of 3.75 × to 384 ×. The microscopes can also be equipped with remote control pedals for zoom function.

The Microvid **computer microscopy module** from Wild Leitz turns microscope eyepieces into a computer screen, so that data can be reflected into the microscope and photographed together with the image *(Reader Service No. 107)*. The Microvid projects text, graphics and

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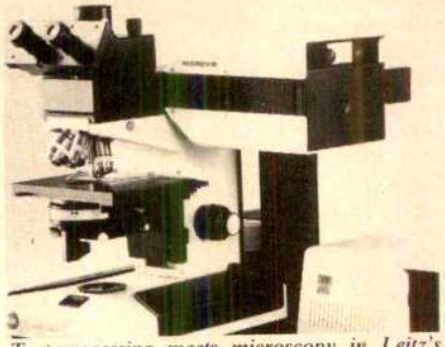
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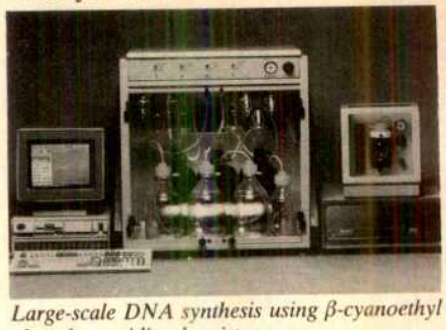


Text-processing meets microscopy in Leitz's Microvid computer microscopy module.

measuring scales onto the viewing image of any modern Leitz microscope through an optical link. The system fits onto the side of Leitz microscopes for measuring and marking the original microscope image, outside the darkroom. The Microvid's software performs measurements of area and perimeter, speed and time. Text can be input at any position on the image, in two different character sizes. Self-drawn and standard graticules can be faded in over the microscope image for sizing and precise counting.

DNA details

MilliGen/Biosearch, a division of Millipore Corporation, has announced the introduction of its model 8800 **large-scale DNA synthesizer** to meet the demand for



Large-scale DNA synthesis using β-cyanoethyl phosphoramidite chemistry.

large quantities of DNA in diagnostic and therapeutic applications *(Reader Service No. 108)*. The model 8800 is a modular, benchtop instrument controlled by an IBM PC workstation which MilliGen/Biosearch says can synthesize from 30 to 300 μmol of DNA at one time. The system has a batch reaction design which relies on base- dependent coupling and deblocking protocols. The fully programmable synthesizer has software which performs reagent calculations and has notebook capabilities. The standard protocols are based on β-cyanoethyl phosphoramidite chemistry, but the system can be adapted for other chemistries as well.

DNAStar, Inc. has a **compact disk** containing the Brookhaven Library of Protein Structures which allows researchers to view the structures of protein molecules in three dimensions by wearing stereo glasses *(Reader Service No. 109)*. The company developed the X★RAY disk as a less inexpensive adjunct to high-performance molecular workstations for protein chemists, and as an aid for students wishing to familiarize themselves with 3-dimensional protein structures. DNAStar says X★RAY rapidly paints the detailed composite molecular structures in colour stereo views on the screen of any IBM PC/AT/XT or PS/2 equipped with an EGA or VGA colour graphics monitor. The displayed molecules can be fully rotated with and without side chains, and can be printed out on a dot matrix or laser printer, or an HP Plotter.

Biotix, Inc. is introducing a **high-speed DNA synthesizer** at the San Francisco exhibition that it says is capable of pro-



Five-minute oligonucleotides with 99 per cent purity from Biotix.

ducing high-purity oligonucleotides with 99 per cent efficiency in coupling cycles of under five minutes *(Reader Service No. 110)*. Both the one- and two-column models of the synthesizer are fully automated and user programmable. The synthesizer has a 200-mer capacity, and Biotix guarantees that it will synthesize oligonucleotides of up to 125 bases. The $14,950 single-column instrument comes complete with an IBM- compatible PC.

Perkin-Elmer Cetus is now producing **recombinant *Taq* DNA polymerase** for use in the polymerase chain reaction (PCR) for DNA amplification *(Reader Service No. 111)*. The company's AmpliTaq



Recombinant Taq *DNA polymerase from Perkin-Elmer Cetus comes singly or as a kit.*

DNA polymerase is genetically engineered and purified using an enzyme process that Perkin-Elmer Cetus says removes unwanted thermostable nucleases. The company says further that studies comparing AmpliTaq with native *Taq* DNA polymerase show that the thermostability and specificity of the recombinant enzyme is equivalent to that of the native form in the PCR process. AmpliTaq DNA polymerase is available separately in 250-unit, 0.05 ml microtubes, or as part of Perkin-Elmer Cetus' GeneAmp DNA amplification reagent kit. The company says researchers who are currently using native *Taq* DNA polymerase can switch to AmpliTaq without modifying their protocols.

Gels and more

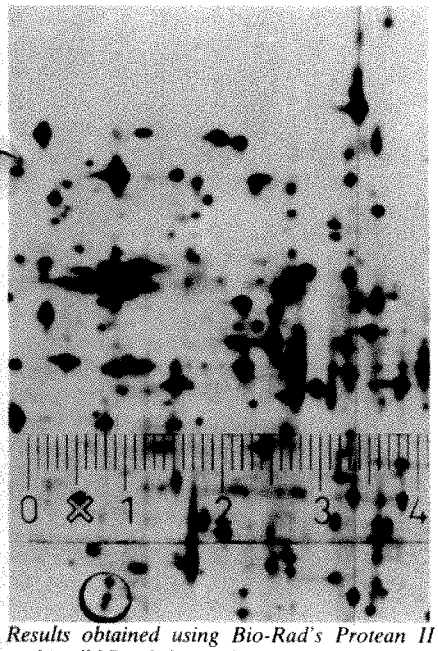
The new model 270A **analytical capillary electrophoresis** system from Applied Biosystems couples the separation power of electrophoresis with microprocessor-controlled instrumentation *(Reader Service No. 112)*. The company says the system makes the fast analysis of a wide variety of molecules routine and minimizes the pre-run preparation time traditionally required by standard electrophoretic protocols. Unlike traditional gel methods, the model 270A automates the electrophoretic separation of molecules in free solution within small-bore capillary tubes, without a gel matrix or denaturant. The entire procedure is automated by an integral, menu-driven system controller.



Applied Biosystems brings analytical capillary electrophoresis to the lab with the model 270A.

Sample loss is virtually eliminated due to the small amount of sample required for analysis, says ABI, and the overall reagent and buffer consumption is extremely low because a small-bore capillary is used.

Bio-Rad has recently released an updated version of its Protean II multi-cell **two-dimensional gel electrophoresis** apparatus *(Reader Service No. 113)*. The improved system comes with a more efficient cooling system that permits higher power settings with minimal diffusion. The gels are first attached to the three central cooling cores of the multi-cell, for uniform heat removal. Two



Results obtained using Bio-Rad's Protean II multi-cell 2D gel electrophoresis setup.

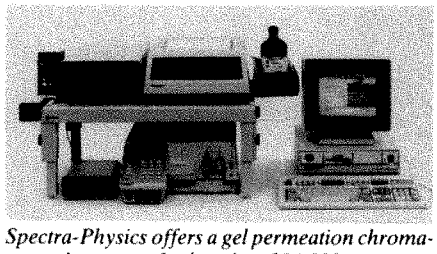
aluminium cooling coils coated with an electrically non-conductive material that exhibits high thermal conductivity are then placed in the lower buffer chamber to chill the second dimension running buffer. The gels are run submerged in the chilled running buffer for optimal heat dissipation. Two new detergents that eliminate equilibration and overnight focusing steps — CHAPS and NONIDET P-40 — are available for use with the system, which comes complete with a step-by-step protcol manual.

Amersham International now offers its Hyperfilm-MP and Hyperfilm- β max high-performance **autoradiography films** in rolls of 20 cm × 25 m *(Reader Service No. 114)*. The larger films are designed for applications which require longer pieces of film than the sizes currently available in sheets, such as DNA sequencing, where users are frequently forced to splice two pieces of film together. For applications such as whole body autoradiography and thin-layer chromatography which require less than one conventional sheet of film, the rolls are more economical. Amersham's Hyperfilm-β max roll film is intended for high-speed, high-resolution direct autoradiography of compounds labelled with ^{14}C, ^{35}S or ^{125}I. The Hyperfilm-MP film is for use with ^{3}H, ^{14}C, ^{32}P or ^{125}I, and can be used with intensifying screens or fluorography.

Molecular Dynamics says its new HRDI Autophoresis 1000A instrument will do for electrophoresis what the telescope did for astronomy *(Reader Service No. 115)*. The company's astronomical claims centre on the high-resolution dynamic imaging (HRDI) properties of the instrument, that allow the detection of bands in electrophoresis gels in real time, without staining. The Autophoresis system not only eliminates gel staining steps and autoradiography, but also allows the user to determine when the compounds of interest are sufficiently separated. Because diffusion is virtually eliminated, Molecular Dynamics says smaller compounds — such as peptides and other molecules which either diffuse rapidly or do not stain well — can be detected. The HRDI optical technique is related to the phase contrast imaging used in microscopy. The gel is illuminated by a HeNe laser light source which projects the image of the gel onto a screen. Portions of the gel which do not contain sample bands appear as dark areas on the screen because light which passes straight through the gel is absorbed by a mask. Bands in the sample bend the light so that it bypasses the mask and is focused on the viewing screen.

Separation and analysis

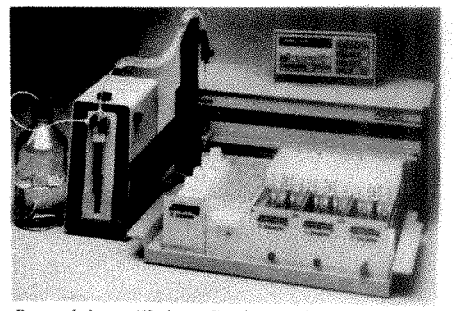
The GPC System for **gel permeation chromatography** from Spectra-Physics



Spectra-Physics offers a gel permeation chromatography system for less than $14,000.

includes modular LC hardware, comprehensive software and a selection of columns *(Reader Service No. 116)*. The system consists of two parts: the chromatography hardware including a pump, detector and column oven which the company says is capable of delivering performance repeatability in the one per cent range; and the multi-tasking software which runs on either a Spectra-Physics WINner+ or a ChromStation/2 workstation/integrator. The GPC sofware performs standard operations, collects and stores raw data, calculates molecular weight distribution, plots raw chromatograms, and calibrates using narrow range, broad range or universal standards. Time/area slices or complete reports can be stored automatically, and reports can be customized to fit specific requirements. Once the data are stored, annotated replots of original chromatograms can be produced with drawn-in baseline. The complete hardware system costs $11,300 (US) and the accompanying sofware, $1,250 (US).

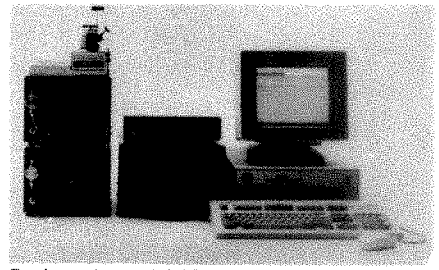
Gilson Medical Electronics has a **sample preparation system** that both cleans a sample automatically and injects the prepared sample into an on-line HPLC system without supervision — the



Busy labs will benefit from Gilson Medical Electronics' ASPEC system.

ASPEC system *(Reader Service No. 117)*. Gilson says the ASPEC system is compatible with most standard 100-mg and 500-mg disposable extraction columns and can process up to 108 complex samples. ASPEC can be used automatically to perform extraction, elution and concentration steps, or for liquid handling operations such as transfers and dilutions. The software is flexible so that operators may select the proper volume and flow rate of each solvent at each extraction step, or program a unique operational sequence. Applications of the ASPEC system include liquid-solid extractions of hormones and drugs in biological fluids, nucleic acids and lipids in tissue/cell extracts, and pesticides in soil and water.

The model 338 **gradient liquid chromatograph** from Beckman Instruments links reliable pumps with programmable UV-visible detectors and provides single-point instrument control *(Reader Service No. 118)*. The system's model 110B pump flow rate range of 0.01–10 ml min^{-1} can be increased to 28 ml min^{-1} with the addition of preparative pumpheads, so that only one system is needed for analytical, high-

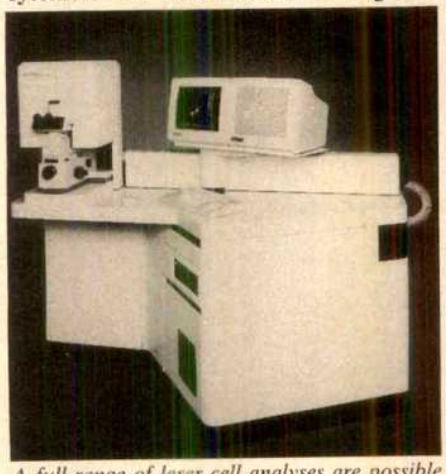


Beckman's model 338 gradient liquid chromatograph.

speed and semipreparative chromatography. The variable wavelength Beckman UV-visible 166 detector offers sensitivity down to 0.001 AUFS with low refractive index response and stable baselines, says the company. Results can be optimized automatically through the built-in time programming of absorbance range, wavelength and auto zero. The model 338 system's controller is a NEC lap-top computer, which is linked through an analogue interface to the pump and digitally to the detector module. For more powerful data handling, the system can be used with a desktop PC running Beckman's System Gold software.

A look inside

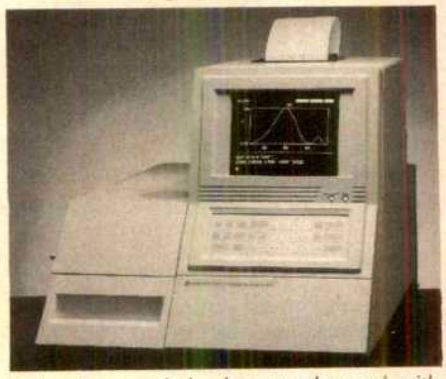
Meridian Instruments has recently introduced its ACAS 570 **interactive laser cytometer** for adherent cell sorting and



A full range of laser cell analyses are possible using Meridian's ACAS 570.

quantitative fluorescence analysis *(Reader Service No. 119)*. A focused laser spot provides the capability to use interactive methods, as well as to perform automated quantitative fluorescence imaging and analysis, confocal microscopy, and the measurement of intracellular ions such as calcium and *p*H. Meridian's instrument can be used for optical sectioning, intercellular communication studies, the real-time measurement of cellular dynamics, and the isolation of mutant or transfected cells for propagation or analysis using a unique patented process called "cookie cutter". The ACAS 570 uses an argon ion laser and dual photometric detection system for single or simultaneous dual parameter or ratio measurements on single cells or entire cell populations. Meridian says the system provides optimal sensitivity and quantitation of fluorescence measurements with minimal photobleaching.

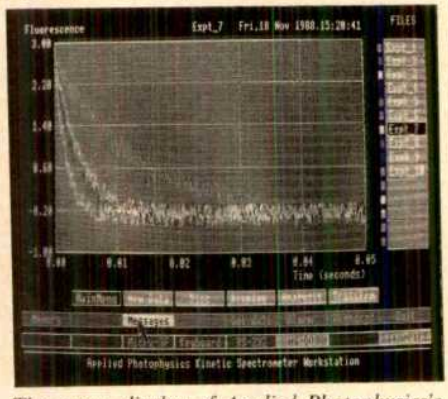
Hitachi Instruments has a new **intracellular calcium measurement system** designed for use with Quin, Fura or Indo dyes — the model F-2000 *(Reader Service No. 120)*. A sample introduction system combined with report generation for both tabulated and graphical data provide a



Quin, Fura or Indo dyes can be used with Hitachi's new calcium measurement system.

complete package for the application. The F-2000 features a wavelength slew rate of 200 nm s^{-1} so that a maximum number of data points can be measured during the analysis. Hitachi says the horizontal beam geometry provides a four-fold increase in sensitivity and allows samples of 0.6 ml to be measured routinely in a standard 1 cm cuvette.

Applied Photophysics will be launching two new **spectrometry instruments** at the San Francisco meeting *(Reader Service No. 121)*. The company's model SF.17MV microvolume biocompatible stopped-flow spectrofluorimeter requires just 100 µl of each reactant to prime the sample flow circuit. Comprehensive absorption and fluorescence capabilities, facilities for anaerobic and large-ratio mixing, and a high-performance 32-bit RISC-processor spectrometer workstation



The screen display of Applied Photophysics's stopped-flow spectrofluorimeter.

are provided as standard. Applied Photophysics' model RX.1000 rapid mixing spectrometer accessory can be fitted to any absorbance or fluorescence spectrometer that has a regular 1-cm cuvette holder. The company says the accessory can reduce the shortest half-life measurable on a spectrometer from minutes down to less than one second. Designed to satisfy the users of precious and sensitive chemical and biological samples, the accessory also features an anaerobic capability and large-ratio mixing, and offers comprehensive thermostatting facilities.

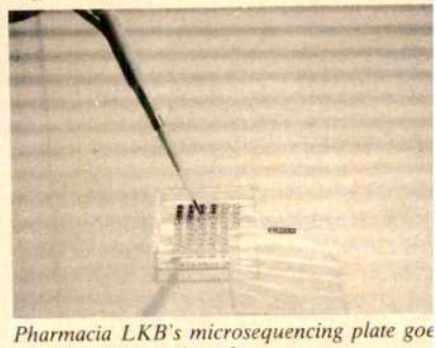
Enzymes, etc.

The radiochemicals division of ICN Biomedicals, Inc. has expanded its series of ^{125}I-labelled immunoglobulins with the addition of **goat anti-mouse IgM ^{125}I** *(Reader Service No. 122)*. The antibodies are prepared by immunizing the appropriate host with a purified immunoglobulin from normal serum. The hosts are bled, and the antisera is pooled and then affinity-purified to ensure species specificity. The purified antibodies are then labelled by a mild chloramine-T method and further purified by HPLC. Each batch is tested by measuring its binding to the appropriate immunoglobulin.

Seikagaku America offers a host of products for **cell culture** and manipulation, including enzymes for mucopolysaccharide lysis, cell wall lysis, glycosidases, reverse transcriptase, two inhibitors of myosin light chain kinase and protein kinase activators *(Reader Service No. 123)*. The company's Cellmatrix medium is a collagen gel for culturing cells and tissue. Seikagaku says Cellmatrix is superior to rat-tail collagen because it forms gels more rapidly and reproducibly yields higher strength gels. The medium is useful not only for coating tissue culture surfaces, but for collagen gel matrix culture. Cells can be grown inside the gels to mimic three-dimensional growth *in vivo*.

Pipettes and plates

Pharmacia LKB offers a **microplate for DNA sequencing** which will allow researchers to perform all of the sequencing reactions in a handy sample holder, with no need for tubes and racks *(Reader Service No. 124)*. The company's microsample plate is specifically designed for Sanger reactions: each of its 60 wells accommodates 15 µl and reagents can be added directly to the mixture. The new DNA strand is separated by simply floating the plate on the surface of boiling



Pharmacia LKB's microsequencing plate goes from bench, to bath, to freezer.

water. No evaporation of sample is required. After the gels are loaded, the sample holder can be covered with the lid provided and placed in the freezer.

Eppendorf has a **pipette** for dispensing solutions directly from tall bottles *(Reader Service No. 125)*. The company's Multipipette can now be coupled with its Combilong adaptor to reach into the depths of large bottles. The Combilong consists of an aspirating tube and chamber which has to be filled with solution. The liquid level in the chamber remains constant: the Combitip of the Multipipette takes the liquid directly from the reservoir. The 30-cm long Combilong can be shortened to any length.

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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BIOTECHNOLOGY

CODON is a research and manufacturing, product-oriented biotechnology corporation specializing in the development and manufacture of high value-added recombinant protein products. We are rapidly expanding, and focused at finding novel solutions to today's most complex medical and veterinary conditions.

Employment opportunities at Codon cover a wide range of disciplines, including protein chemistry, molecular biology, immunology, tissue culture, process development, fermentation, biochemical engineering and quality assurance. Most positions require a minimum of a baccalaureate degree and laboratory experience in the above or allied fields.

To find out how you may fit into this exciting, intellectually stimulating environment, please send your resume and letter outlining area of interest to: **CODON, Human Resources Dept. 572, 213 E. Grand Ave., South San Francisco, CA 94080.** Equal Opportunity Employer

Our location, just south of San Francisco, California, places us in one of the major biotechnology centers in the country and affords close contact with prime educational institutions such as Stanford University, The University of California, San Francisco, and The University of California, Berkeley.

CODON

(NW3186)A

UNIVERSITY OF LIVERPOOL

Department of Physiology

Postdoctoral Senior Research Assistant

A three year post is available to investigate a second messenger system recently discovered to operate in the brain in association with excitatory amino acid receptors of the NMDA type (see Nature 336, 385-388, 1988). The work will be multidisciplinary in approach and will be carried out on isolated CNS cells and brain slices. Applications from individuals whose primary expertise is in electrophysiology, pharmacology, biochemistry or cell biology are all welcome.

The project is funded by the MRC at an initial salary within the range £9,865-£11,680 per annum. Informal enquiries may be made to Dr. John Garthwaite. Tel. 051-794 5329.

Applications, together with the names of 3 referees, should be received as soon as possible by **The Director of Staffing Services (AS). The University, P.O. Box 147, Liverpool, L69 3BX,** from whom further particulars may be obtained. Quote ref. RV/238/N.

An Equal Opportunity Employer (8179)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London) DEPARTMENT OF CLINICAL PHARMACOLOGY SENIOR RESEARCH OFFICER In Cellular Biochemistry/Immunology

Applications are invited from post-doctoral scientists to work on the signal-transduction system involved in IgE-mediated response of macrophages. The events appear complex, and involve the CD23 antigen which fulfils a receptor role for both low MWt B Cell Growth Factor and IgE. Funding is for 3 years from the Asthma Research Council.

Salary will be on the scale: £9865-£15720 plus £1650 London allowance a year.

Application forms and further particulars are available from the **Personnel Officer, Royal Postgraduate School, 150 Ducane Road, London W12 0NN** (tel: 01-740 3204) quoting ref: AEW3.

Closing Date: 9th February 1989. (8168)A

POSTDOCTORAL POSITIONS/MOLECULAR BIOLOGY

Positions available immediately for independent and highly-motivated molecular biologists to join a group of immunobiologists studying molecular and cell biological aspects of T cell activation, including transmembrane signalling and the role of receptors, protein kinases and G proteins. Experience in molecular biology techniques (library construction, gene cloning and sequencing, mRNA expression and cDNA transfection) is essential. Well-qualified candidates with keen interest in this area should apply to:

Dr. Amnon Altman
Research Institute of Scripps Clinic
10666 North Torrey Pines Road/IMM3
La Jolla, California 92037
(619) 554-8602

An Affirmative Action/Equal Opportunity Employer (NW3187)A

FACULTY, MOLECULAR VIROLOGY

The Department of Ophthalmology of Washington University School of Medicine invites applicants for a position at the Assistant or Associate Professor level to join a dynamic core research faculty with interests in infectious and inflammatory diseases that may affect the eye. The successful Ph.D. or M.D. candidate will have an active research program in molecular virology, experience with one or more ocular pathogens (herpesviruses, adenovirus, HIV), and an interest in combining basic scientific methodology to the study of ocular disease processes. Previous specific experience in ophthalmic research is not required. Start-up funds, laboratory and a competitive salary are available. Submit curriculum vitae and three letters of reference to:

Henry J. Kaplan, M.D.
Chairman, Department of Ophthalmology
Washington University School of Medicine, Box 8096
660 South Euclid Avenue
St. Louis, MO 63110 (NW3182)A

FACULTY POSITIONS

SLOAN-KETTERING INSTITUTE

CELL BIOLOGY AND GENETICS PROGRAM

The Sloan-Kettering Institute invites applications for several tenure-track junior and senior faculty positions in the Cell Biology and Genetics Program. This research program is one of four in the Institute, and is undergoing a major expansion in research at the molecular level on the areas of positive and negative growth control, differentiation, cell structure, intercellular recognition, and morphogenic pattern formation. The Cell Biology Program and the Molecular Biology Program at the Sloan-Kettering Institute are jointly relocating to a new research building which also houses advanced facilities for protein and nucleic acid microchemistry, transgenic mice, and automated mammalian cell micromanipulation. The faculty members of the Cell Biology and Genetics Program are also affiliated as faculty with the Cell Biology Graduate Program at Cornell University Medical School. The positions offered are highly competitive with regard to start-up funds, laboratory space, salary and housing.

Eligible candidates should have a doctoral degree, post-doctoral experience and demonstrate potential and ability to develop an independent, imaginative research program. Formal applications are invited from junior as well as established investigators. Applications should include a curriculum vitae, a list of publications, a summary of research activities and research plan, and the names of three or more references. Applications should be addressed to: **Dr. Joan Massague, Cell Biology and Genetics Program, Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021.**

(NW3199)A

STRUCTURAL BIOCHEMISTRY FACULTY POSITION UCLA

The Department of Chemistry and Biochemistry and the Molecular Biology Institute have a **TENURE-TRACK OPENING** in structural biochemistry. The position probably will be filled at the assistant professor level, but applications for a more senior position will also be considered. We are seeking a candidate who will establish a strong and innovative research program in the area of biological structure, although highly exceptional applications in other areas will be considered. Teaching responsibilities will include undergraduate biochemistry and participation in graduate courses appropriate to the appointee's interests. Candidates should submit curriculum vitae and statement of research plans, and should have three letters of recommendation sent to:

Dr. David Eisenberg
Department of Chemistry & Biochemistry
University of California
Los Angeles, CA 90024–1569
by April 15, 1989

The University of California is an Equal Opportunity/Affirmative Action Employer, and encourages applications from women and minority candidates. (NW3224)A

Animal Physiology/ Molecular Biology/ Tissue Culture

STAFF SCIENTISTS

TKT Inc. is a newly established biotechnology company dedicated to the development of innovative technologies for the treatment of human disease, emphasizing gene therapy. We are seeking Ph.D. level scientists to conduct research on animal physiology, mammalian gene expression, and regulation of cell growth. Experience in one of the following areas is essential:

Animal physiology: development of animal model systems; mammalian endocrinology and metabolism. Experience in small animal surgery is essential.

Molecular biology: recombinant DNA technology; structure and function of mammalian genes.

Tissue culture: establishment, maintenance and transfection of mammalian cell lines.

Excellent location in Cambridge, Massachusetts, competitive salaries and benefits, and an opportunity for equity participation in a challenging environment. Send resume and list of three references to: **TKT Inc., Suite 154, 295 Cambridge St., Boston, MA 02114.**

(NW3217)A

Oregon State University

Assistant or Associate Professor Laboratory for Nitrogen Fixation Research Oregan State University

A 12-month tenure-track position is available at the rank of Assistant or Associate Professor with responsibility to lead a vigorous research program on the molecular biology and physiology of symbiotic nitrogen fixation and to teach a graduate course in the area of expertise. Requirements include a Ph.D. degree, postdoctoral experience, a strong background in biochemistry or plant physiology and in molecular biology, and an established record of research productivity. Applicants should submit a curriculum vitae, statement of research and teaching interests, list and samples of publications, and names of four references to: **Search Committee for Nitrogen Fixation Position, Department of Botany and Plant Pathology, Oregon State University, Cordley Hall 2082, Corvallis, OR 97331-2902.** Closing date: March 31, 1989. Position available: September 1, 1989. OSU is an AA/EOE and complies with Section 504 of the Rehabilitation Act of 1973. OSU has a policy of being responsive to the needs of dual-career couples. (NW3214)A

RESEARCH ASSOCIATE

MOLECULAR BIOLOGY OF CYTOKINES

Laboratory of Medical Biochemistry
The Rockefeller University

Independently conduct molecular and genetic research in ongoing projects to characterize macrophage-derived inflammatory mediators and immunomodulatory monokines. The highly motivated scientist we seek will have demonstrated competence in applied molecular biology including cDNA cloning and expression, gene regulation, PCR applications and hybridization techniques. Must demonstrate competence to coordinate all molecular biological approaches in the efforts of a multidisciplinary team exploring the biochemistry, cell biology, immunology, in vivo functions and clinical importance of several newly-discovered cytokines.

Salary commensurate with research experience and leadership abilities. The Rockefeller University is an Equal Opportunity/Affirmative Action Employer and invites applications from all qualified individuals. Please contact:

Anthony Cerami, Ph.D.
Professor and Head,
Laboratory of Medical Biochemistry
The Rockefeller University
1230 York Avenue
New York, NY 10021

THE ROCKEFELLER UNIVERSITY

(NW3204)A

Cell Biologist
Biophysicist
Structural Biologist
Molecular Biologist

The Pennsylvania Muscle Institute and the University of Pennsylvania School of Medicine seek outstanding candidates for two tenure track positions in the area of fundamental cardiovascular or motility research. One position will be in the field of molecular genetics of contractile proteins.

The other position is in the broad area of cell biology, biophysics and/or structural biology of muscle. These positions are primarily aimed at, but not restricted to, the Assistant Professor level. Appointment will be made in the department appropriate to academic interest.

Applicants should send CV and research statements and the names of three references to: **Yale E. Goldman, M.D., Ph.D., Director of Pennsylvania Muscle Institute, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6085.**

Equal Opportunity/Affirmative Action Employer. (NW3052)A

Career Opportunities in Chemical Information

Molecular Biology
(German, French, Russian)

Microbiology
(German, French, Russian)

General Biochemistry
(German, French, Japanese, Russian)

Analytical Biochemistry
(German, French, Japanese, Russian)

Pharmaceutical Chemistry
(German, French, Japanese, Russian)

Medicinal Chemistry
(German, French, Japanese, Russian)

The continuing growth of CAS's information services has created openings for full-time chemical scientists to be involved in the extraction and analysis of chemical information. An advanced degree in one of the scientific areas listed above plus skills with one of the languages listed is required. However, you do not need prior work experience in this activity.

CAS is located in Columbus, Ohio, a growing and dynamic metropolitan area with excellent housing, cultural, recreational and transportation facilities. Our modern offices, situated in a campus-like setting, provide a professional and comfortable working environment.

CAS provides a complete benefits package, which includes flexible working hours, tuition reimbursement, generous vacation and a range of employer-paid insurance programs.

Applicants must be located in and be eligible to work in the U.S. If you meet our specific qualifications, you can apply by sending your resume (indicating position desired) and salary requirements to:

Chemical Abstracts Service
Employment Department-B
P.O. Box 3012
Columbus, Ohio 43210 (NW3174)A

A Division of the American Chemical Society

An Equal Opportunity Employer M/F

UNIVERSITY OF LIVERPOOL
Interdisciplinary Research Centre for Surface Science

Experimental Officer/Senior Experimental Officer

Applications are invited for the position of Experimental Officer/Senior Experimental Officer in the Interdisciplinary Research Centre in Surface Science. The successful applicant will be attached to the Interfaces and Semiconductor Surface Chemistry project of the IRC.

The appointee will be responsible for the support of existing equipment and the installation and development of new equipment. There will also be opportunities to take part in the planning and execution of the research programme. Applicants should have a degree or equivalent qualification. Experience of surface science instrumentation would be an advantage.

This is a permanent position. Initial salary on the scale for Experimental Officer £8,675-£13,365 per annum or Senior Experimental Officer £12,145-£15,720. Informal enquiries may be made to Dr. P. Weightman. Applications, including c.v. and the names of three referees, should be received not later than 17 February 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69,** from whom further particulars may be obtained. Quote ref. RV/243/N.

An Equal Opportunity Employer (8178)A

UNITED MEDICAL AND DENTAL SCHOOLS
LECUTRESHIP IN THE DIVISION OF PHARMACOLOGY & TOXICOLOGY

Applications are invited for a Lecturer in the Division of Pharmacology and Toxicology. The post will be for three years in the first instance with a possible extension to five years.

Teaching commitments will be primarily to pre-clinical medical students and there may be some administrative duties. The lecturer will be expected to pursue research in pharmacology. No particular line of research is required but the Division has particular interests in psychopharmacology (Guys Campus), electrophysiology of muscle, central transmitters and retinal degenerative diseases (St Thomas's Campus). The new lecturer will be based at the campus most appropriate for his/her area of research.

Intending applicants should have a first degree in medicine, pharmacology or related subject, a PhD or MD and must have the ability to present lecturers, communications etc in a clear and interesting way.

Further information may be obtained from Professor M J Neal, Division of Pharmacological Sciences, UMDS, St Thomas's Campus on 01-928 9292 Ext 2080. Salary will be on the lecturer scale Grade 'A' £9,260-£14,500 or Grade 'B' £15,105-£19,310 plus London Weighting Allowance, depending on age and qualifications.

Applications, with full curriculum vitae (six copies) and the names of two referees, should be sent to the **Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** quoting reference STH/PHAR 283 by 15 January 1989. (8180)A

CHAIRPERSON

DEPARTMENT OF STOMATOLOGY
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
SCHOOL OF DENTISTRY

Full-time position as Chairperson of the Department of Stomatology which includes programs in oral biology, oral medicine, oral pathology, periodontology, and oral radiology. Responsibilities include providing academic and administrative leadership in research, teaching, patient care and community and university service for undergraduates, predoctoral and postdoctoral students, and faculty.

Demonstrated administrative experience and academic qualifications (D.D.S., M.D., Ph.D. or equivalent degree) for a senior professorial rank are required. Salary commensurate with qualifications and experience. Applications from women and minorities are encouraged. The University of California, San Francisco is an equal opportunity, affirmative action employer.

Please send curriculum vitae and the names of three individuals familiar with your work to **Dr Leonard B. Kaban, Chair, Search Committee, in care of: Office of the Dean, School of Dentistry, Box 0430, UCSF, San Francisco, California 94143.**

Applications should be submitted by February 28, 1989.

(NW3171)A

ERATO

Exploratory Research for Advanced Technology

RESEARCH DEVELOPMENT CORPORATION OF JAPAN

RESEARCH ENGINEERS AND SCIENTISTS: You are invited to COME TO JAPAN to do research at the forefront of basic science and engineering. ERATO, a program fully funded and administered by the Research Development Corporation of Japan (JRDC), a statutory corporation of the Japanese government, has been inviting non-Japanese researchers to Japan since 1981. ERATO is now seeking researchers for the following projects:

SAKAKI QUANTUM WAVE (1988-1993) – **Solid physicists, solid state chemists,** and **semiconductor engineers** will research the fabrication and behavior of quantum wells and wires, structures with features smaller than the electron's mean free path.
Project Director: Professor Hiroyuki Sakaki, Research Center for Advanced Science and Technology, University of Tokyo

MASUHARA MICRO PHOTOCONVERSION (1988-1933) – **Spectroscopists, applied physicists, electronic engineers, physical chemists, catalysis chemists, polymer chemists, synthetic organic chemists, materials scientists,** and **bioengineers** will research the use of lasers to drive, measure, and control pico-second chemical reactions in micrometer-size volumes.
Project Director: Professor Hiroshi Masuhara, Kyoto Institute of Technology

MIZUTANI PLANT ECOCHEMICALS (1988-1993) – **Natural products chemists, organic chemists, analytical chemists, biochemists, molecular biologists, genetic engineers, botanists, plant physiologists, microbiologists, entomologists, zoologists,** and **ecologists** will research the ways in which plants use chemical substances to regulate surrounding plants and animals.
Project Director: Professor Junya Mizutani, Hokkaido University, Sapporo

NISHIZAWA TERAHERTZ (1987-1992) – **Solid state physicists, electron physicists, electronic engineers, solid state chemists,** and **communications engineers** are researching the fabrication, behavior, and measurement of semiconductor devices for operation at terahertz frequencies.
Project Director: Professor Jun'ichi Nishizawa, Tohoku University and Semiconductor Research Institute, Sendai

FURUSAWA MORPHOGENE (1987-1992) – **Protein chemists** are invited to join embryologists, developmental biologists, and other protein chemists in researching the identification and expression of gene groups which control morphogenesis with special focus on the early differentiation and development of germ cells and on the fundamental processes of gastrulation.
Project Director: Dr. Mitsuru Furusawa, Deputy General Manager, Dai-ichi Seiyaku Research Institute, Tokyo

KUNITAKE MOLECULAR ARCHITECTURE (1987-1992) – **Synthetic chemists, polymer chemists, surface chemists,** and **solid state physicists** are researching the architecture and function of self-assembling organic systems – their molecular design and synthesis, the introduction of function, and the creation of three-dimensional architectures.
Project Director: Professor Toyoki Kunitake, Kyushu University, Fukuoka

HOTANI MOLECULAR DYNAMICS ASSEMBLY (1986-1991) – **Biophysicists, biochemists,** and **molecular and structural biologists** are researching the ways in which supramolecules perform functions such as self-organization, energy conversion, and information processing.
Project Director: Dr. Hirokazu Hotani, Associate Professor, Kyoto University

ERATO RESEARCHERS BENEFIT FROM

Well-funded, multidisciplinary research
Long-term positions – allowing for significant, in-depth research
Industry-competitive salaries
Sharing of patent rights and profits
Assistance for Japanese language learning (ability in Japanese not required)
Cooperative agreements with industry to allow corporate researchers to join

For an evaluation of ERATO, see "JTECH (Japanese Technology Evaluation Program) Report on ERATO," National Science Foundation, Dec. 1988 – available from NTIS, 5285 Port Royal Road, Springfield, Virginia 22161 USA, Telephone (703)487-4826.

Please send your resume and list of publications to ERATO, c/o

ERATO Overseas Representative **ISTA**

International Science & Technology Associates, Inc.
11 Beatrice Drive, Bryn Mawr, Pennsylvania 19010-2301 USA
Telephone: (215)527-4538, Facsimile: (215)527-2041

(W5771)A

CHAIRPERSON

DEPARTMENT OF BIOCHEMISTRY

emple University School of Medicine is seeking an individual with emonstrated administrative skills and an outstanding record of chievement in research and teaching to succeed the present chairan. The candidate should demonstrate a commitment to excellence research, medical education, and graduate training. The departent currently has excellent research and graduate programs, and fective collaborative arrangements with the Fels Institute for Cancer esearch and Molecular Biology and the Thrombosis Center at emple University. As one of the five medical schools in the Philadelhia area, there are excellent opportunities for collaborative ograms with other institutions, the Fox Chase Cancer Center, and veral major pharmaceutical companies in the Delaware Valley and rrounding area.

pplications and nominations together with curriculum vitae may be nt to: **John J. O'Neill, Ph.D., Chair, Search Committee, Department Pharmacology, Temple University School of Medicine, 3400 North road Street, Philadelphia, PA 19140.**

n Equal Opportunity/Affirmative Action Employer. (NW3200)A

PENN STATE

FACULTY POSITION

PROTEIN X-RAY CRYSTALLOGRAPHY

Applications are invited for a tenure-track faculty position at any level in protein x-ray crystallography. The position will be available in either the Department of Chemistry, the Department of Molecular and Cell Biology, or as a joint appointment in these departments or with Penn State's Biotechnology Institute. Applicants, with a Ph.D. degree, should have the background, motivation, and ability to develop vigorous, productive research programs and to be excellent teachers.

Include a detailed curriculum vitae, statement of research interests, and up to three recent reprints, and have three letters of reference sent directly to: **The Search Committee, Department of Chemistry, 152 Davey Laboratory, Box N, University Park, PA 16802.** Deadline for receipt of applications is 15 March 1989, or until a suitable candidate is identified.

Equal Opportunity Employer. Women and minorities encouraged to apply. (NW3166)A

POSTDOCTORAL POSITIONS IN AUDITORY PHYSIOLOGY AND NEUROANATOMY

Institutional Postdoctoral Fellowships are available in the Johns Hopkins Center for Hearing Sciences. Current research areas include: hair cell physiology, stimulus coding and signal processing in the peripheral and central auditory system, neuroanatomy of the cochlear nucleus, cellular biophysics and electrophysiology in cochlear nucleus and psychoacoustic measures of hearing in animals.

Send vita and names of three references to: **Director, Center for Hearing Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.**

The Johns Hopkins University is an equal opportunity/affirmative action employer. (NW3194)A

POSTDOCTORAL POSITIONS

available to study biology and biochemistry of basic fibroblast growth factor (bFGF). Ongoing projects include regulation of bFGF synthesis and expression, autocrine activities of bFGF, biochemistry of growth factor interaction with extracellular matrix, and characterization of the bFGF receptor. Experience in biochemistry or molecular biology preferred. Send curriculum vitae and names of three references to **Dr. Daniel B. Rifkin or Dr. David Moscatelli, Department of Cell Biology, New York University Medical Center, 550 First Ave., New York, NY 10016.** (NW3178)A

RESEARCH POSITIONS

available immediately for (two) recent PhD's with strong background in molecular biology, recombinant DNA studies, and/or biochemistry. Projects involve studies on regulation of cellular and viral oncogenes and their products in mammary cells. Related areas of research include the expression of HIV genes under hormonal control. Technical help is available. Positions could lead to nontenure track faculty position within 12 months and the candidates will be given opportunity in developing independent research programs. Salary is negotiable. Please submit curriculum vitae and names of three references to: **Professor N. H. Sarkar, Head Laboratory of Molecular Virology, Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912-2100.** EOE/AAP. (NW3208)A

POSTDOCTORAL POSITION IN CELLULAR NEUROBIOLOGY

Position available immediately to study physiology and pharmacology of synaptic events in the spinal slice preparation and molecular mechanisms of tachykinin actions. Project involves the use of single-electrode voltage-clamping and whole-cell voltage-clamping with a patch electrode. Send curriculum vitae, statement of research interests, and names of three references to:

Mirjana Randic,
Department of Veterinary Physiology and Pharmacology,
College of Veterinary Medicine,
Iowa State University,
Ames, IA 50010, U.S.A.

Closing Date: March 1, 1989. (NW3193)A

ASSISTANT PROFESSOR

MOLECULAR NEUROBIOLOGY

A tenure-track position is available in a growing interdisciplir neuroscience research program between the Departments of Pec rics and Anatomy. Preference will be given to applicants who h demonstrated expertise in applying molecular biology to the foll ing areas of CNS research: oncology, development, immunolog genetics. The successful applicant will be expected to conduct active research program which complements ongoing resea efforts, to obtain extramural support and to participate in the med school teaching responsibilities of the Department of Anatomy.

Please submit curriculum vitae, statement of research goals, names and addresses of four references to: **Shirley Ann Gilm Ph.D., Professor and Chairman, Department of Anatomy (#5 University of Arkansas for Medical Sciences, 4301 West Markh Little Rock, AR 72205-7199**

The University of Arkansas for Medical Sciences is an Equal Oppo nity/Affirmative Action Employer. (NW3219)

SENIOR STAFF INVESTIGATOR

Applications are invited for a full-time resea position in the Renal Research Program, Departm of Medicine. The position involves research in broad areas of extracellular matrix biology. Applica must have demonstrated strong motivation tow creative research and ability for independent wo Candidates must possess a Ph.D. or M.D. degree a substantial experience in the molecular biology a biochemical aspects of collagen/proteoglyc biosynthesis. Excellent facilities and support available. Please submit curriculum vitae and repri of most recent significant publications to: **Mr. Da Shepherd, CFP-514, Henry Ford Hospital, 2799 W Grand Boulevard, Detroit, MI 48202.** (NW3172)A

UNITED MEDICAL AND DENTAL SCHOO GUY'S CAMPUS — CLOSE TO LONDON BRIDGE

LABORATORY SCIENTIFIC OFFICER or RESEARCH ASSISTANT (pending on experience and qualifications) in Bacteriology, with rel ence to an immunological research project into prevention of colc sation of Oral Streptococci and Anaerobic Bacteria. The appointm is for two years in first instance.

Salary in range: £6,190 + £1,267 London Weighting (LSO) — £13, + £1,650 London Allowance (Research Assistant), according to perience and qualifications.

For further information please contact Miss Lesley Bergmeier (01-4 7600 ext 2965).

Applications, with full curriculum vitae and names of two referees, **Staffing Officer, UMDS, Lambeth Palace Road, London, SE1 7E** quoting Ref: G/IMM/286. (8192)A

UNITED MEDICAL AND DENTAL SCHOOL GUY'S COMPUS — CLOSE TO LONDON BRIDGE

RESEARCH ASSISTANT IN MOLECULAR SCIENCE required for clo ing and sequencing of microbial surface proteins or T cell surfa receptors. Graduate or Postdoctoral scientists may apply if they ha experience in molecular biology or protein chemistry.

Salary, according to qualifications and experience, in range £8,675 £14,500 plus £1,650 London Allowance. Appointment is for thr years.

For further information, please contact Professor Thomas Lehner Dr Charles Kelly — 01-407 7600 ext 2960/2965.

Applications, with full curriculum vitae and names of two referees, **Staffing Officer, UMDS, St Thomas's Campus, Lambeth Palace Roa London SE1 7EH,** quoting Ref: G/IMM/284. (8191)A

NWO

The Laboratory for Space Research, part of the Space Research Organisation of the Netherlands (SRON), has a tenure tracked position for an

astronomer

available to support their work on infrared spectroscopy and submm observations.
SRON is founded within the framework of the Netherlands Organisation for Scientific Research (NWO). The Laboratory is located at the University of Groningen.

The astronomer should be oriented towards theory and interpretation and should also be able to guide observational programmes. The work should be mainly motivated by the data bases already acquired by the Laboratory, and to be obtained in the future: IRAS and the IRAS low Resolution Spectrometer data (special processing is done in Groningen), data obtained with the JCMT at Hawaii and in the future ISO spectrometer data.

The candidate is expected to have close contacts with the data analysis group and with the Kapteyn Astronomical Institute. He will also play an important role in the ISO observations programmes, particulary in spectroscopy.

The salary depends on experience and ranges from ƒ 3.867,— to ƒ 6.908,— a month.

Candidates are invited to apply for this position. Letters, together with three letters of recommendation, should be addressed to the personnel manager, Mrs. W. Lawerman, SRON, Beneluxlaan 21, 3527 HS Utrecht, the Netherlands. Reference number ROG 88/6. More information on the job can be obtained from the Director of the Laboratory, Dr. Th. de Graauw (tel.: 31-50-634074). The closing date for application is march 1, 1989.

(W5790)A

UNIVERSITY OF OXFORD
Departmental Demonstrator in Pharmacology

The Department of Pharmacology wishes to appoint a Demonstrator to teach CNS pharmacology. The person appointed should have the appropriate research experience and would be expected for the first three years to join an existing research group, either in the Department or in the MRC Anatomical Neuropharmacology Unit. Projects available are: neurochemistry and neurophysiology of modulatory mechanisms in the basal ganglia (Dr. S.A. Greenfield) or the pharmacology and chemical neuroanatomy of cerebral cortex, hippocampus, basal ganglia or medullary cardiovascualr centres (MRC Unit).

The salary is on the scale from £8675 to £12150 p.a. according to age and the post is for three years in the first instance. The appointment may be extended for a further maximum period of three years in which case the Demonstrator would be allocated independent research space.

Applicants must have experience in the appropriate techniques in neuroscience (e.g. intracellular recording or neuroanatomy) and should apply by March 1st 1989 to **Professor A.D. Smith, Department of Pharmacology, South Parks Road, Oxford OX1 3QT, from whom further particulars may be obtained. (Tel: 0865- 275162; FAX: 0865-275161).**

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER (8201)A

POSTDOCTORAL/RESEARCH SCIENTIST

Ph.D. in Biochemistry or Biology. To study adipocyte differentiation at the cellular and molecular levels. Work will include purification of cell produced factors influencing differentiation, study of their expression and action during differentiation at the molecular level. Positions available in June and September 1989. Please send curriculum vitae and three letters of references to **Dr. Ginette Serrero, c/o Personnel Office, W. Alton Jones Cell Science Center, 10 Old Barn Road, Lake Placid, NY 12946.**

Please refer to Job No. 88-21. The Cell Science Center is an Equal Opportunity/Affirmative Action Employer. (NW3170)A

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AUSTRALIAN NUCLEAR SCIENCE & TECHNOLOGY ORGANISATION

RESEARCH FELLOW
MATERIALS SCIENCE/ELECTRON MICROSCOPY

Position No. 762

Research Scientist
$A31,003-$A38,045 per annum

Senior Research Scientist
$A39,349-$A45,177 per annum

A vacancy currently exists within the Advanced Materials Program of the Australian Nuclear Science and Technology Organisation (ANSTO) for a research fellowship within the Electron Microscopy Group. The successful applicant will be expected to interact fully with other scientists in the program and will have opportunity to participate in collaborative programs with industry. Current projects include:

- Phase development and crystal chemistry of Synroc, a titanate ceramic for nuclear waste encapsulation.
- Studies of structural changes in minerals and synroc during weathering or hydrothermal dissolution.
- Radiation damage in metamict minerals and neutron irradiated ceramics.
- Advanced ceramics for biomedical and electrical applications e.g. high Tc superconductors, proton conductors etc.
- Remnant life studies of steels.

The fellow will also be encouraged to develop his/her own research projects and/or industrial collaboration, provided such interests fall within the Programs general aims.

The Advanced Materials Program has one JEM 2000 FX TEM fitted with a Gatan TV system and a conventional Si/Li ED spectrometer; and a second JEM 2000 FX fitted with a wide bore pole piece and a thin window detector. Other facilities include a JEOL JSM 6400 SEM, a JEOL JXA 840 analytical SEM, a computer controlled Phillips PW 1050 diffractometer, a Siemens D 500 automatic XRD unit, optical microscopes, a Zeiss quantitative image analysis system, mechanical testing equipment and an extensive ceramics processing laboratory.

The Organisation utilises a salary system based on personal merit for this category of staff. Progression to higher grades is possible as experience, skills, knowledge and personal contributions to the Organisation's programs increase.

Salary depending upon qualifications and experience will be within the above range.

Knowledge, qualifications and Skills
Applications are sought from electron microscopists **and** from materials scientists with experience in analytical TEM techniques. The skills sought include experience with HRTEM, ATEM, image processing, image simulation, materials fabrication, metallurgy, crystal chemistry, mineral dissolution/alteration, structure/property relationships, radiation damage, and electroceramics.

Applicants should have a PhD or equivalent in relevant physical sciences and extensive experience with transmission electron microscopy.

At least two Professional referees should be nominated. It is intended that the skills and experience of the successful applicant will complement those of existing staff.

Employment will be for a term of 3 years, with Australian Government superannuation benefits available.

Contact Officer for further information is Dr K Smith telephone (02) 543 3505 or Fax (02) 543 5097.

Closing date for applications is 17 February, 1989, although applications received after that date may be considered. It is hoped that the successful applicant would begin duties on or before 1/4/89.

Application forms and selection criteria may be obtained by telephoning (02) 543 3064 or writing to the Recruitment Officer, Australian Nuclear Science and Technology Organisation, Private Mail Bag 1, Menai, N.S.W. 2234, Australia, quoting the above position number.

ANSTO IS AN EQUAL OPPORTUNITY EMPLOYER.

SW3175

NEUROBIOLOGIST

Two postdoctoral positions are available immediately in a multidisciplinary laboratory for individuals interested in pursuing studies on the function of astrocytes. One position involves examination of astrocytic responsiveness using a video-based imaging system to monitor changes in intracellular ions via fluorescent probes. The second position involves isolation of pharmacologically-distinct subpopulations of astrocytes and examination of factors released from astrocytes following receptor stimulation. Individuals interested should have a strong background in biochemistry and pharmacology.

Send curriculum vitae to **Dr. Ken D. McCarthy, Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7365.**

Equal Opportunity Employer. (NW3212)A

UNIVERSITY OF WARWICK

Senior Technician

The Department of Biological Sciences has a vacancy for a technician to organise and co-ordinate the technical facilities of a section of the Virology Research Group. The person appointed will also be actively involved in research, under the direction of Professor N J Dimmock, on projects which currently range from viral immunology to molecular cloning.

Applications are invited from persons who are suitably qualified and experienced in one or more of these areas and who have an interest in technical administration. The post has prospects for regrading if proposed developments materialize.

Salary on the Technician Grade 5 scale: £8088–£9549 pa.

Application forms from the **Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523685)** quoting Ref No 24/4T/88/14 (please mark clearly on envelope). Closing date for applications 2 February 1989.

AN EQUAL OPPORTUNITIES EMPLOYER (8194)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
DEPARTMENT OF HAEMATOLOGY
MRC/LRF LEUKAEMIA RESEARCH UNIT

RESEARCH TECHNICIAN required in Leukaemia Cell Biology Laboratory. The work in this laboratory is directed towards immunological control of leukaemia cell growth.

The position includes responsibility for long term storage of blood and bone-marrow in liquid nitrogen, preparation of culture media and reagents, and maintenance of cell lines. General duties will include maintenance of laboratory supplies.

Applicants should have good general laboratory experience, preferably with a haematology/immunology background. For further information please call Dr Julie Adams 743-2030 Ext 3237.

Salary on range £7415-£10148 plus £1267 London Allowance pa.

Application forms and further particulars are available from the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road London W12 0NN** (tel: 01-740 3204) quoting reference AFB3.

Closing Date: 7th February 1989 (8169)A

UNITED MEDICAL AND DENTAL SCHOOLS
GUY'S CAMPUS — CLOSE TO LONDON BRIDGE

LABORATORY SCIENTIFIC OFFICER

required to join a team working on DNA sequencing and cloning of microbial proteins and T cell surface receptors. Experience in molecular biology or microbial biochemistry is desirable.

Salary, according to qualifications and experience, in range £6,190 to £12,214 plus £1,267 London Weighting. Appointment is for three years.

Applications, with full curriculum vitae and names of two referees, to **Staffing Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH**, quoting Ref: G/IMM/285. (8193)A

UNIVERSITY OF GLASGOW
Department of Veterinary Pathology
Postdoctoral and Graduate Research Assistants
Retrovirus Vectors and Transgenic Animals

Applications are invited from postdoctoral and graduate molecular biologists for an AFRC funded project aimed at developing retrovirus for the production of transgenic domestic animals.

These positions are available for three years from February 1989 on the 1A Scale for postdoctoral applicants (£11,680 – £15,720) and the 1B scale for graduates (£8,675 – ££11,680).

The successful candidates will join an active group involved in the production of transgenic animals resistant to viral infections and they will be part of a department which has a major interest in the molecular biology of human and animal retroviruses.

Postdoctoral applicants must have an extensive working knowledge of recombinant DNA procedures. Experience of work on the molecular biology of viruses, particularly retroviruses, would be an advantage. Graduate applicants should have a degree in virology, molecular biology, or a related discipline and must have had experience of recombinant DNA techniques.

Applicants should send a CV, including publications and the names of two referees, to **Prof. D. Onions, Director, Leukaemia Research Fund Centre, Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH, from whom further details may be obtained.** (8176)A

THE ROYAL VETERINARY COLLEGE
University of London
Department of Veterinary Basic Sciences
POSTDOCTORAL RESEARCH ASSISTANT

An appointment is available to assist in a project to determine the molecular and cellular response to physical loadbearing in bone tissue. Experience in molecular and cellular biology is an advantage but not essential.

The project is supported by The Wolfson Foundation and the appointment will be for one year in the first instance with extension possible up to three years.

SALARY on the University Research Grade 1A scale, ranging from £11,515 to £17,370 per annum. Starting salary according to age and experience. Eligibility for Universities' Superannuation Scheme.

Further details may be obtained from Professor L E Lanyon (phone 01-387 2898).

Applications with a cv and the names of two referees to the **Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU.**

CLOSING DATE FOR APPLICATIONS: 23 February 1989 (8234)A

Medical Research Council
Radiobiology Unit
Cell and Molecular Biology Division

Molecular/Cellular Biologist

Applications are invited for a non-clinical scientific post which may be filled by either a SHORT TERM appointment of five years or a CAREER appointment according to the age and experience of the successful candidate. A career appointment will be tenable at this Unit in the first instance. The successful candidate will join a group studying molecular and cellular aspects of DNA repair and radiation mutagenesis, chromosome replication and aberration induction and the biophysics of radiation damage. Excellent research facilities are available and the Unit also includes groups studying mammalian genetics, radiation leukaemogenesis, cancer therapy and radiation chemistry. The postholder will be required to continue and expand the current programme on DNA repair and mutagenesis; in addition, a career appointee will develop his/her own programme of research and promote studies involving molecular biology.

Salary on scales equivalent to those for University non-clinical academic staff: Grade 2/1: £11,070–£19,310 or for Senior Grade up to £22,910 pa. Pension provided. The Unit is situated in a pleasant rural area equidistant to Newbury and Oxford. Hostel accommodation available.

Please send applications, including full c.v., publication list and the names and addresses of 2 referees, to the Personnel Officer, MRC Radiobiology Unit, Chilton, Didcot, Oxon, OX11 0RD. Please telephone Dr. John Thacker (0235 834393) for further information. Closing date: 24th February 1989.

The Council is an Equal Opportunity Employer (8226)A

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF CLINICAL CHEMISTRY
WOLFSON RESEARCH LABORATORIES
LECTURESHIP (NON-CLINICAL) IN CLINICAL CHEMISTRY

pplications are invited for this post of Lecturer (non-inical). The successful candidate will have a thorough aining and achievement in research and the ability to itiate and develop a research programme which comple-ents and extends the scientific interests of the Wolfson esearch Laboratories and the University School of Med-al Science. These include the development and clinical pplication of biochemical markers of malignant and ndocrine disease using molecular, cellular and novel nmonassay techniques. The Lecturer will contribute to esearch training and teaching of students, especially at ostgraduate level and formal qualifications in clinical hemistry are not essential.

alary will be within Lecturer A or B scales plus super-nnuation. It is anticipated that an experienced candidate f high calibre will be appointed on the Lecturer B scale urrently £15,105 – £19,310 pa).

urther particulars may be obtained from: **The Senior ssistant Registrar, University of Birmingham Medical chool, Vincent Drive, Birmingham B15 2TJ. Tel: 021 414 049 Ext 3377.**

o whom applications, including curriculum vitae and ames and addresses of three referees should be sent not ter than: February 24 1989. (8188)A

IVERPOOL SCHOOL OF TROPICAL MEDICINE
(University of Liverpool)
Department of Parasitology
OST DOCTORAL RESEARCH ASSISTANT

pplications are invited for the above MRC/EEC funded post which is ailable immediately for a period of 2 years with the possibility of tension for a 3rd year. The successful candidate will be involved imarily in applied immunological research on human hydatidosis d cysticercosis. The project has strong collaborative links in Kenya, ina and Mexico. Experience in both immunology and molecular ology would be advantageous. Starting salary within the range 865-£10460 pa. Applications with full c.v. and three references ould be sent by 10 February 1989 to **Dr P S Craig, Dept of Parasitology, verpool School of Tropical Medicine, Pembroke Place, Liverpool 5QA.** The Liverpool School of Tropical Medicine is an Equal Oppor-nity Employer. (8205)A

UNIVERSITY OF SUSSEX
POSTDOCTORAL POSITION IN MOLECULAR GENETICS

plications are invited from candidates interested in membrane otein structure and assembly for the above MRC-funded post.

e successful candidate will use a newly developed gene fusion stem to analyse the membrane organisation and mechanism of embrane insertion of selected eukaryotic membrane proteins. Prev-us experience in rDNA technology would be useful, but is not sential.

e project is funded for 3 years on the RA1A scale (£9,865 – £11,070 a.) and is available to start immediately.

.v. with the names and addresses of two referees, should be sent to **Jenny Broome-Smith, Microbial Genetics Group, School of ological Sciences, University of Sussex, Falmer, Brighton BN1 G, E. Sussex.**

EQUAL OPPORTUNITY EMPLOYER (8233)A

PLANT BREEDING INTERNATIONAL
NEW OPPORTUNITIES WITHIN OUR EXPANDING SCIENCE GROUP
ASSISTANT MANAGERS/RESEARCH ASSISTANT

Two assistant managers and one research assistant are required for two different teams, one working on the molecular biology of plant viruses, and the other on the molecular biology of potato nematode disease resistance. The assistant managers will have line management responsibilities.

Qualifications: Either a HND, BSc or equivalent, and laboratory experience in molecular biology and/or biochemistry, extensive in the case of assistant managers, together with a high degree of care and reliability in experimental work.

For further particulars telephone Cambridge 840411 ext 307, or write to the **Personnel Officer, PBI Cambridge Ltd, Maris Lane, Trumpington, Cambridge CB2 2LQ quoting Ref: SCI/42/43.**

An Equal Opportunities Employer (8231)A

RESEARCH FELLOW
$A31,003-$A38,045

DIVISION OF RADIOPHYSICS
MARSFIELD, NSW AUSTRALIA

THE DIVISION: The Division of Radiophysics, a Division within the Institute of Information and Communications Technologies, provides research, development and consulting for signal and image processing, VLSI design, gallium arsenide design, millimetre and microwave technology receivers, earth station and satellite antennas. It is the host institution of the Australia Telescope, and has a highly regarded international reputation in the area of radio astronomy.

THE GROUP: The Signal and Imaging Technology Group within the Division is currently involved in the areas of VLSI design, ultrasonics, radar systems, tomographic imaging, medical electronics, special purpose high-speed processors for image processing, and other aspects of signal processing. The potential of using new technology together with theoretical research in signal and image processing techniques forms much of the work within the group. Much of this work is done in cooperation with Australian industry, although some of the work is applied to the radio astronomy research being undertaken by the Division.

THE JOB: The appointee will undertake research associated with the application of modern interactive image processing technology to novel geophysical techniques being developed for the coal industry. This project is being carried out under contract to the Australian Coal Industry Research Laboratories and involves collaboration with Macquarie University. The appointee will be required to liaise closely with geophysicists from these groups in the evolution of techniques for both measurement and processing. To this end it will be necessary for the appointee to participate in underground surveys.

THE PERSON: Applicants should hold a PhD degree or equivalent qualification in electrical engineering or geophysics, with demonstrated ability in original research. Extensive experience with numerical computation is essential. Expertise in modern image reconstruction technology, familiarity with geophysical practice and some knowledge of geology would be of considerable benefit.

CONDITIONS: Appointment will be for a term of 2 years, with Australian Government superannuation benefits available.

ENQUIRIES: Prospective applications are invited to contact Mr Glynn Rogers or Dr John O'Sullivan (02) 868 0222 for further information.

APPLICATIONS: Applications should be submitted by 24th February and should quote reference number A7002. They should provide details of relevant personal particulars, including qualifications and experience. The nomination of at least two professional referees should also be included. Applications should be addressed to:

The Chief
CSIRO Division of Radiophysics
PO Box 76, Epping, NSW 2121
AUSTRALIA

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W5772)A

Advanced Drug Delivery Research

Head of Molecular Cell Biology

Horsham, West Sussex, U.K.

The Advanced Drug Delivery Research Unit of Ciba-Geigy Pharmaceuticals is working at the forefront in the design of methods for achieving the site-specific delivery of conventional and genetically defined drugs. A senior position has become available within the Unit for an experienced Cell Biologist. This person will act as the scientific leader of a multi-disciplinary project team comprised of up to 20 staff working on achieving the epithelial and endothelial transport of macromolecules.

This is an exciting career opportunity for a creative and effective senior scientist with a renowned track record in cell and molecular biology and possessing proven team leadership skills. The successful applicant will preferably have had at least 10 years' post-doctoral experience as well as industrial and international experience.

The remuneration package offered reflects the seniority of this position, and includes comprehensive relocation assistance where appropriate.

Candidates wishing to discuss this position in confidence may contact Professor Eric Tomlinson, Head of the Advanced Drug Delivery Research Unit at Horsham (ext. 3638).

Applicants should send a full CV quoting ref: V40 to the Personnel Department, Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex RH12 4AB. Telephone: (0403) 50101.

CIBA–GEIGY

(8196)A

UNIVERSITY OF SOUTHAMPTON
CRC MEDICAL ONCOLOGY UNIT
(Southampton)

GROWTH FACTOR PROJECT

Experienced **(post-doctoral)** cell biologist/biochemist required in group studying role of poly-peptide growth factors on the growth and dissemination of tumours. Appointment in the first instance for 3 years within Research Fellow Grade 1A (Salary in the range of (£9,865 – £15,720).

Applications to Ms J A Doyle, Staffing Section, The University, Southampton SO9 5NH.

Further details may be obtained from Professor Peter Alexander, tel. 0703 777222 Extn. 4296 or 4297. Please quote reference 189/JAD. (8203)A

NATIONAL HEART AND LUNG INSTITUTE
BROMPTON HOSPITAL
RESEARCH ASSISTANT
(Pre Doctoral 1B)

Applications are invited for the post of Research Assistant in the Department of Paediatrics Respiratory Medicine Division.

A life science graduate is required, ideally with experience of HPLC techniques and immunoassays, to work on a Ministry of Agriculture Fisheries and Food supported project on the mechanisms and mediators involved in food and food additive intolerance.

The post is funded for 2 years in the first instance. The appointee will be expected to register for a higher degree.

Starting salary up to £10,460 pa. plus £1,450 London Weighting.

Information and details may be obtained by direct contact with Dr. J.O. Warner in the National Heart and Lung Institute, Brompton Hospital on 01-352 8121 Ext. 4804.

Applications with full CV and names of two referees should be sent to the **Personnel Manager, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, quoting ref BPA4.** (8235)A

THE UNIVERSITY OF ADELAIDE
South Australia

invites applications from women and men for the follo position:

RESEARCH ASSOCIA IN THE DEPARTMENT BOTANY
(Ref. 6688)

An appointee is sought for a ject entitled "Foliar Physiogn and Taxomomic Compositio Northern Australian Rainfo and the Taphonomy of Resu Leaf Beds" which is being dire by Dr D C Christophel. The ject is documenting relation between canopy components, litter and stream deposited litt various rainforest types Queensland, Australia, to a fossilisation potential.

Applicants should have a hi degree in a relevant field an perience in foliar physiogn taphonomy, tropical angios taxonomy or tropical ecology would be an advan The appointee should be prep to spend 2-3 months per year i field.

The position is available mediately for one year in the instance with a possibilit appointment for a further pe The appointee would be expe to commence by 10 July 1989.

Further information concer the duties of the position ma obtained from Dr D C Christo telephone (08) 228 5710.

Information about the ge conditions of appointment ma obtained from the Senior A tant Registrar (Personnel) a University, or from Appointm (35924), Association of C monwealth Universities, Gordon Square, London W 0PF.

Salary per annum: A$26,61 — A$30,360.

Applications, in Dupli quoting reference number and giving full personal partic (including whether candi hold Australian permanent dency status), details of acad qualifications and names addresses of three referees sh reach the Senior Assistant R trar (Personnel) at the Unive of Adelaide, GPO Box 498, laide, South Australia, 5 Telex UNIVAD AA 89141, simile 61 8 224 0464 not later th March 1989.

The University reserves right to make enquiries of person regarding any candi suitability for appointment, n make an appointment or appoint by invitation.

The University of Adelaide equal opportunity empl (W5779)

GBF

Gesellschaft für Biotechnologische Forschung mbH —
ıe National Research Centre for Biotechnology, Braunschweig, Federal Republic of Germany

vites applications from experienced immunologists and molecular ologists for two

Postdoctoral Fellow

ɔsitions in the Division of Microbiology.

ıe Environmental Microbiology Group of the Division of Micro- ology is seeking to expand its expertise in microbial ecology by ɔpointing two postdoctoral fellows capable of applying and further eveloping modern immunological (Post 1–No. 16/89) and DNA/RNA ıquencing (Post 2–No. 17/89) methods to the study of microbial ɔmmunity structure and function of biotechnologically important ɔosystems. Part of the work will involve the analysis of any commu- ty changes resulting from pollution or the addition of large quanti- es of pure cultures. Appointees will work closely with other embers of the Environmental Microbiology Group (microbiolo- sts, biochemists, molecular biologists, ecologists) interested in the odegradation of environmental pollutants.

pplicants for Post 1 should be immunologists or have a strong ımunological background and be familiar with monoclonal anti- ɔdy techniques. Applicants for Post 2 should have a background in icrobiology, molecular genetics, biochemistry or chemistry and ave experience with nucleic acid sequencing. For both posts appli- ınts should have an excellent research and publication track record ıd a strong interest (preferably experience) in microbial exology. ıe posts, which are initially available for two years but may be tended by mutual consent, are available immediatley.

pplications, which should be sent with the announced number to e **Personnel Department, GBF, Mascheroder Weg 1, D-3300 raunschweig, FRG**, should include a complete c.v., list of publica- ɔns, reprints/preprints of the most important publications, a escription of past and present research activities, and the names and ddresses of three persons willing to provide critical references.

(W5776)A

POSTDOCTORAL POSITIONS

olecular Genetics of Drosophila available in either of two labora- ries to study chromosome organization and its relationship to ɜne function. Possible projects include sequence organization ıd position effects of chromosome termini (R. Levis) and regula- ɔn of nested genes (S. Henikoff). Send CV and addresses of ree references to either individual at:

Fred Hutchinson Cancer Research Center,
1124 Columbia Street,
Seattle, WA 98104.

HCRC is an AA/EEO, M/F/H employer. (NW3192)A

POSTDOCTORAL POSITION

MOLECULAR VIROLOGY

position is open to carry out research on the mecha- sm of retroviral replication using site directed muta- ɜnesis.

andidates must have a Ph.D., preferably with some ɔstdoctoral experience and are expected to join a nall research team. Research grant and travel money ɜe available as well as technical help. The position is ır a three years period.

alary will be commensurate with background and (perience and will range from $35,000.–$45,000–/year.

ɜnd a curriculum vitae and the names of three refer- ɜs to **Professor P.-F. Spahr, Department of Molecular ology, University of Geneva, 30, quai Ernest- nsermet, 1211 Geneva 4, Switzerland.** (W5792)A

INSTITUTE OF PSYCHIATRY
DEPARTMENT OF NEUROPATHOLOGY

SENIOR CHIEF MLSO

(GRADING SUBJECT TO REVIEW)

Applicants are invited from suitably qualified MLSO staff for the positon of Senior Chief Medical Laboratory Scientific Officer in the Department of Neuropathology, Institute of Psychiatry.

Applicants should have a knowledge of histopathological techniques, and experience in neurohistopathology is preferable.

Since the applicant will be in charge of the technical management of a large, multidisciplinary department with varied clinical services and extensive research programmes, managerial skills are essential.

Salary scale in the range £13,354–£16,248 pa plus £1,201 London Weighting. The grading of this post is subject to review in accordance with the terms of the Whitley Council agreement on revised salary and grade structure.

Further details may be obtained from Professor P Lantos 01-703 5411 ext 3272.

Application forms are available from the **Assistant Secretary's Office, Institute of Psychiatry, De Crespigny Park, London SE5 8AF** quoting ref PL/

Closing date: 17th February 1989 (8167)A

PROFESSIONAL VACANCIES

RESEARCH SCIENTIST/ SENIOR RESEARCH SCIENTIST (Several Positions)

$A30,100-$A43,801 p.a.

DIVISION OF APPLIED PHYSICS
SYDNEY, NSW, AUSTRALIA

Fields: Physics, electrical and mechanical engineering, and related fields.

General: The Division of Applied Physics undertakes research in Physics and Engineering and collaborates with industry to develop new or improved products and processes. It also maintains Australia's primary standards of measurement for a wide range of physical quantities and undertakes research related to the development of new or improved standards. Most of the Division's staff, numbering about 300 in all and including some 130 professional scientists, are located at a modern laboratory in the Sydney suburb of Lindfield. The research of the Division is carried out in five programs: Electrotechnology; Applied Electricity and Magnetism; Plasmas, Thin Films and Thermometry; Acoustics and Mechanics; and Optical Technology. Vacancies exist in these programs and we seek applications from experienced scientists who believe they have attributes that would enable them to make innovative contributions both to existing and future projects.

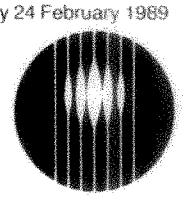
Duties: To undertake research within one of the programs listed above. Current projects include: superconductivity at low and high temperatures and related device applications; systems for the transport of bulk materials; fabrication and application of new magnetic materials; electricity supply technology; plasma applications, e.g. in materials processing; surface modification and thin film technology; applied ultrasonics; optical information processing; fabrication and coating of high-quality optical components; and several projects on measurement science, related to mechanical, electrical, acoustical, optical and temperature measurement technology, both in the standards context and for specific industrial applications. Further information may be obtained by phoning Dr John Collins on (02) 467 6324.

Qualifications: Ph.D or equivalent in a relevant field of science or engineering plus a proven record of achievement in independent research indicating self motivation and adaptability. Experience working in or with Industry would be an advantage.

Tenure: Indefinite with Australian Government Superannuation benefits available.

Applications: Applications should be submitted by 24 February 1989 and quote reference No. AS88/22. They should include relevant personal particulars, including qualifications and experience and the names of at least two professional referees, and be addressed to:

The Chief,
CSIRO Division of Applied Physics,
P.O. Box 218, Lindfield, NSW 2070
AUSTRALIA



CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W5774)A

GBF

Gesellschaft für Biotechnologische Forschung mbH —
The National Research Centre for Biotechnology, Braunschweig, Federal Republic of Germany

seeks two experienced

IMMUNOLOGISTS/CELL BIOLOGISTS

for the Division of Microbiology.

Two posts are available, one which is tenured for a senior scientist (No. 14/89), the other of which is available for two years (renewal possible) for a postdoctoral fellow (No. 15/89).

Requirements and Responsibilities
Candidates for both posts should possess a doctoral degree in medicine of science and have extensive experience (documented by excellent publications) in immunology and with modern immunological methods. The persons appointed will camp out fundamental research on intestinal immune and non-immune defences against microbial infections. They will interact closely with microbiologists and geneticists of the Molecular Medical Mircobiology group that are studying pathogenesis mechanisms of and developing vaccines against bacterial intestinal pathogens. The more senior appointee will additionally be responsible for setting up and supervising a monoclonal antibody facility for the Department of Microbiology, and thus extensive experience in tissue culture methods and hybridoma techniques is important. Appropriate resources will be made available for this facility.

Applications, which should be sent with the anounced number to the **Personnel Department, GBF, Mascheroder Weg 1, D-3300 Braunschweig, FRG**, should include a complete c.v., list of publications, reprints/preprints of the most important publications, a description of past and present research activities, and the names and addresses of three persons willing to provide critical references. (W5777)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
DEPARTMENT OF MEDICAL MICROBIOLOGY

Position with honorary College appointment for Post Doctoral molecular biologist to collaborate with virologists in research on drug resitant HIV. Experience preferred in application of PCR and DNA sequencing. Academic associates in discipline already present. Salary available for immediate appointment with three years MRC support.

Apply to Professor George Gee Jackson or Dr John Oxford, Department of Medical Microbiology, The London Hospital Medical College, Turner Street, London E1 2AD (Tel: 01-377 7141). (8185)A

Christie Hospital & Holt Radium Institute

PATERSON INSTITUTE FOR CANCER RESEARCH

Department of Carcinogenesis

RESEARCH SCIENTIST

Experienced post-doctoral scientist required to participate in one or more aspects of the group's work on molecular mechanisms of cancer causation and treatment, with particular emphasis on DNA damage and repair.

She/he would be expected to generate new research programmes within the framework of the group's interests. Experience in gene transfer techniques, especially the use of retroviral vectors or transgenics, or methods for the detection of DNA damage in human tissues could be relevant.

MRC conditions of employment and remuneration depending upon age and experience.

Please send CV and an outline of research interests to Dr P J O'Connor, Head, Carcinogenesis Department, Paterson Institute for Cancer Research, Christie Hospital & Holt Radium Institute, Manchester M20 9BX, U.K.

FUNDED BY THE CANCER RESEARCH CAMPAIGN (8221)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following position:

FACULTY OF SCIENCE
FIXED-TERM LECTURER IN CHEMISTRY

Duties will include teaching to undergraduate and graduate students and conducting research in chemistry. The appointment is for 3 years in the first instance with the possibility of extension. The appointment is in inorganic chemistry, preferably with a biological interest, although other areas will be considered. It is expected that an appointment will be made mid-year. For further information contact the Head of the Department of Chemistry, Dr Ben Selinger (062) 492391 or Dr John Broomhead (062) 492934.

Closing date: *13 March 1989.* Ref. FS.23.12.1

SALARY: A$30,737–A$40,100 p.a. APPLICATIONS should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from Appointments (35929), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER
(W5780)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF MEDICINE
POST-DOCTORAL RESEARCH ASISTANT

Applications are invited for work on toxic oxygen metabolites in vascular disease. Experience in this area is not essential but previous experience in H.P.L.C. techniques is required. The position is for eighteen months in the first instance.

For further particulars please contact Dr M McLaren, Tel: (0382) 60111 Ext 2440.

Applications with CV (2 copies) and the names of 2 referees to the **Personnel Office, The University, Dundee, DD1 4HN** quoting Ref: EST/436/88/N. Closing Date: 9 February 1989.
(8195)A

AGRICULTURAL A
FOOD RESEARCH
COUNCIL
INSTITUTE OF ANIM
PHYSIOLOGY AN
GENETICS RESEAR

BABRAHAM,
CAMBRIDGE CB2 4AT

The Institute proposes to a
an IMMUNOLOGIST
MOLECULAR CELL BI
GIST to develop an indep
research programme in a
of fundamental immun
Research areas of special i
include: the biology of MH
ecules especially in relati
structure, biosynthesis, intr
lar trafficking and the capt
antigenic material; the proc
of antigen, and nature of ar
recognized only by T cells; t
of lymphokines and other g
regulators in the immune res
especially in relation to the
mechanism of action; the c
of antibody class; the nat
specific tolerance including
geny of T and B cells reper
the immunology of epitheli
faces including the functi
gamma-delta cells. Other re
topics may also be considere
in all cases particular empha
be laid on the competence
candidate to develop a re
programme exploiting a vari
cellular, biochemical and m
lar genetic techniques.

The Institute is excepti
well-equipped to support
programme, with on-site pe
and oligonucleotide synthesi
protein sequencing facility,
escence activated cell s
Evans and Sutherland mol
graphics equipment, direct
multi-user VAX network,
erial uniform-field gamma ir
tor and barrier-maintained
small animal unit.

The appointment, which w
tenurable and made at Senior
ary £12,445-£17,032) or Prin
Scientific Officer (Salary £16
£21,737) level depending
experience and qualifications
ries an independent research
get and technical suppor
further appointment at post
toral level may also be consi
in association with the pri
appointment. Non-contrib
Superannuation Scheme. 25
leave and 10.5 public holiday
annum. Further information
be obtained from Dr J C Ho
at the Department of Immun
(Tel: Cambridge (0223) 83
and application forms from M
M Howden, Personnel O
quoting ref: BR2/P by 17/2/89
(8213

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nature
— the professional
choice

BP VENTURE RESEARCH FELLOW

Department of Biological Sciences

UNIVERSITY OF CALIFORNIA, SANTA BARBARA

Professor Ian K. Ross and his associates are investigating novel nuclear/cytoplasmic interactions that regulate differentiation in higher filamentous fungi (Basidiomycetes) under the auspices of the BP Venture Research scheme. The following postdoctoral position is available, offering opportunities to engage in challenging, new, research of considerable significance.

* A molecular biologist with a background in the molecular biology and biochemistry of fungi. Expertise in cloning and transformation techniques required. Experience in protein purification and characterization and/or immunological techniques would be advantageous.

Salaries for the Venture Research Fellow will be (US) $23,000-24,000. The appointment will be for three years initially, but may be renewable.

To apply, send curriculum vitae and three letters of recommendation to Prof. Ian K. Ross, Department of Biological Sciences, University of California, Santa Barbara, 93106, USA.

The University of California is an equal opportunity/ affirmative action employer. Applications from women and members of minority groups are strongly encouraged.

(8174)A

UNITED MEDICAL AND DENTAL SCHOOLS of Guy's and St Thomas's Hospitals

POSTDOCTORAL RESEARCH ASSISTANT

required in the Division of Surgery, St Thomas's Campus, to work on a project investigating the experimental effects of chemical manipulation on carcinogenesis. Candidates should preferably have a higher degree in biology, and a biochemical background would be advantageous.

Salary scale: £9,865 — £12,760 plus £1,650 London Weighting Allowance per annum depending on qualifications, experience and age. Appointment is for one year in the first instance.

For further information, please telephone Mr K G Burnand or Mr D Rutt on 01-928 9292 extns 2117 and 2424 respectively. To apply, please write with full CV and names and addresses of two referees to: **The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH, quoting reference No. STH/SURG/282(A).**

Closing date: 10 February 1989. (8181)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Norfolk Place, London W2 1PG

WOLFSON POST-DOCTORAL RESEARCH FELLOW

required to join group in the Academic Department of Medicine at St. Mary's Hospital Medical School (Head of Department: Professor H.C. Thomas) to work on the molecular biology of delta hepatitis virus. Previous experience in gene cloning and general molecular biology techniques will be an advantage. Post funded by the Wolfson Foundation for a period of three years with starting salary of £13,330 pa inclusive of London Allowance.

Further enquiries to Dr. J. Monjardino (01-725 6381/1606).

Applications in form of full C.V. with names and addresses of 2 referees should be sent to **Personnel Department at above address. Please quote ref. 591.** (8183)A

UBC

The University of British Columbia

FACULTY OF SCIENCE

DEAN

Outstanding candidates are sought for the position of Dean of the Faculty of Science. The new Dean will take office July 1, 1989 or as soon thereafter as possible. The Faculty comprises eleven departments that offer B.Sc., M.Sc. and Ph.D. programs. The B.Sc. can also be earned in selected departments in the Faculties of Arts and of Medicine. There are more than 300 full-time faculty members.

The successful candidate will be a scholar recognized internationally in a field of research that enhances the Faculty. He or she will provide dynamic leadership within the Faculty and play a constructive part in the University's general academic leadership. The Dean will have proven administrative ability and will be dedicated to excellence in teaching.

Applications and nominations should be directed to **Dr. Daniel R. Birch, Vice President (Academic) and Provost, The University of British Columbia, 6328 Memorial Road, Vancouver, B.C. V6T 2B3, no later than March 31, 1989,** and must include a curriculum vitae, a list of publications and the names of at least three referees.

In accordance with Canadian Immigration requirements priority will be given to Canadian citizens and permanent residents of Canada. The University of British Columbia offers equal opportunity for employment to qualified female and male applicants. (NW3173)A

RESEARCH SCIENTIST
MOLECULAR GENETICS AND CARCINOGENESIS
CROSS CANCER INSTITUTE

The Cross Cancer Institute, a comprehensive cancer centre serving the population of northern Alberta, is seeking two molecular/cellular biologists for a tenure track position at the assistant or associate research scientist level. The successful applicants will join a highly interactive multi-disciplinary research group recently established under the aegis of the Alberta Heritage Foundation for Medical Research (AHFMR); the program is devoted to studying the molecular biology of human cancer. Candidates should hold a Ph.D. and/or M.D. degree(s), have relevant postdoctoral experience, and have a demonstrated potential for developing a vigorous and productive research program. While the specific area of research is not as important as excellence, preference will be given to applicants committed to advancing the understanding of human neoplasia in general and cancer cytogenetics, oncogenes/tumor-suppressor genes, control of gene expression, and developmental regulation in particular. Excellent start-up facilities and research support are available, as are opportunities to interact closely with basic and clinical researchers. The position has no formal teaching responsibilities but cross-appointment in the adjoining University of Alberta Medical School can be anticipated. Rank and salary (currently ranging from $38,000 to $57,000 per annum) will be commensurate with qualifications. The successful candidate will be encouraged to obtain AHFMR Scholarship support.

Interested persons should send a curriculum vitae, three reprints, and a synopsis of previous and proposed research, and also arrange to have three references directly forwarded to: **Dr. Malcolm C. Paterson, Director, Molecular Genetics and Carcinogenesis Laboratory, Cross Cancer Institute, 11560 University Avenue, Edmonton, Alberta, Canada T6G 1Z2.** To assure full consideration, applications should be received by May 31. In accordance with Canadian Immigration regulations, priority will be granted to Canadian citizens and permanent residents; however, others are strongly urged to apply. (NW3230)A

UNSW

THE UNIVERSITY OF NEW SOUTH WALES
SYDNEY, AUSTRALIA

Equality of employment opportunity is University policy

SCHOOL OF PHYSICS

RESEARCH ASSOCIATE

(Ref. 027)

DEPARTMENT OF CONDENSED MATTER PHYSICS

A position at the Research Associate level is available for a PhD graduate to work on an Australian Research Council funded project on theoretical physics. The project involves the interactions between nuclear spins in metals and electron spins in semiconductors and the consequent ordered structures. Preference will be given to applicants who have had experience in both analytical and numerical theoretical condensed matter physics.

Excellent conditions offered by the University include a garden setting with easy access to public transport, on campus parking, fully equipped sports centre with olympic pool and gym, shops, banking and credit union facilities.

Salary will be in the range $A26,617 to $A30,360 and a contribution towards initial removal expenses may be required. In addition generous leave, flexible working hours and study leave are provided.

Appointment will be initially for 2 years, with the prospect of extension for a further year.

Further information from Dr D. Miller on (02) 697 4582. (W5773)A

Please submit written application, quoting reference number and the names and addresses of two referees to: The Recruitment Officer, General Staff Office, P.O. Box 1, Kensington, N.S.W. 2033, Australia by 31 March, 1989.

A Cray X-MP supercomputer was installed in Finland at the beginning of 1989. The Centre for Scientific Computing, located in the Finnish State Computer Centre is responsible for operating the computer and developing its environment. Principal users of the computer include universities, the Institute of Meteorolgy, the Finnish State Technical Research Centre, and the Finnish State Computer Centre.

In order to further develop supercomputing in Finland we are now seeking a

SCIENTIFIC DIRECTOR

The scientific director will work directly under the manager of the State Computer Centre. The job contract is for a period of 2-5 years. The duties of the scientific director are the following:

- The main task is to promote supercomputing culture in all ways, both in scientific and applied computing.
- He/she must establish and maintain national and international connections necessary for supercomputing.
- He/she will make proposals to improve the environment of the supercomputer.

Candidates are required to have the competence level of a professor. In particular, international experience, connections to research groups in universities and in industry, and the ability to cooperate are considered beneficial.

For additional information, please contact the chairman of the scientific board, Dr Risto Raitio, Tel. 358-0-134171, network address RAITIO@FINFUN.BITNET.

Send applications to the secretary of the scientific board: Hannu Karttunen, VTKK/TLP 2106, Box 40, SF-02101 Espoo, Finland. Deadline for applications is 6 March 1989. The application should include a Curriculum Vitae, salary requirement and all documents the candidate wants to refer to. (W5796)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

POST-DOCTORAL RESEARCH FELLOW

DEPARTMENT OF IMMUNOLOGY

Applications are invited for a post-doctoral position, supported by an MRC programme grant, to work in the molecular immunology group of Dr Robert Lechler and Professor J R Batchelor. The aim of the proposed research project is to investigate the human T cell repertoire using T cell receptor gene cloning and gene transfection techniques. Applicants with experience in recombinant DNA technology are particularly encouraged to apply. Previous experience in immunoligical research is not essential.

Salary scale will be £11,070–£12,150 plus £1,650 London Allowance a year.

Application forms and further particulars available from the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN (tel: 01-740 3204)** quoting reference AIB2.

Closing Date: 2nd February 1989. (8170)A

RESEARCH OPPORTUNITY IN JAPAN

The Frontier Research Program at the **RIKEN** Institute of Physical and Chemical Research (see Nature 325: 569, 1987) invites applications for postdoctoral positions in the following areas:

— Molecular biology of the immune system and protein chemistry (purification and analysis of proteins) including expression of cloned genes and characterization of gene products at the Laboratory for Molecular Regulation of Aging headed by *Dr. Gabriel Gachelin.*

— Molecular biology of tumor suppresser genes, protein chemistry including characterization of proteins from the brains of Alzheimer patients, and biochemistry, molecular cell biology including eukaryotic cell during proliferation and differentiation at the Laboratory for the Aging Process headed by *Dr. Koiti Titani.*

— Molecular biology of phytochrome, including immunocytochemistry, isolation and analysis of genomic clones *in vivo* footprint analysis and site-directed mutagenetics at the Laboratory for Plant Biological Regulation headed by *Professor Masaki Furuya.*

— Nonlinear optics of organic materials, characterization and device application and organic molecular beam epitaxy technique at the Laboratory for Nonlinear Optics and Advanced Materials headed by *Professor Anthony F. Garito.*

— Protein design and synthesis, and bioelectronics at the Laboratory for the Bioelectronic Materials headed by *Dr. Kevin M. Ulmer.*

Appointments will be initially for one year and may be renewable thereafter.

These Laboratories are located at RIKEN Institute's two establishments in Wako, Saitama and Tsukuba, Ibaraki, on the outskirts of Tokyo.

Candidates should send a detailed curriculum vitae, a list of publications and the names of two individuals who may be contacted for references and summary of research interests to:
Director, Division of the Frontier Research Program,
RIKEN, Wako, Saitama, Japan
Zip code 351–01

(W5785)A

University College, London
Postdoctoral Research Associate
Membrane Biogenesis

A position is available immediately to work with Drs Paul Quinn and David Allan on a project funded by the Wellcome Trust to study the kinetics of membrane biogenesis in relation to the secretory pathway and the control of cell growth. Experience in membrane biochemistry or virology would be an advantage. The post, for one year in the first instance, carries an initial salary of up to £11,680 p.a. (under review) + £1,650 London Allowance.

Applications including c.v. and the names and addresses of two referees should be sent to **Dr P. Quinn, Department of Experimental Pathology, University College and Middlesex School of Medicine, University St, London WC1E 6JJ. Telephone: 01-380 9603 for further information.**

Equal Opportunity Employers (8182)A

UNIVERSITY OF BRISTOL
DEPARTMENT OF BOTANY

RESEARCH ASSISTANT IN PLANT MOLECULAR BIOLOGY

This AFRC-funded post is available immediately for an initial two-year period (with good possibility of extension) to complete cloning an auxin receptor gene. A postdoctoral appointment is envisaged (RA1A scale) but suitably-qualified graduates will also be considered. Address informal enquiries to Dr C M Lazarus (Botany Dept., tel. 0272 303030).

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the **Personnel Office, Senate House, Bristol, BS8 1TH.** Please quote reference A161.

An Equal Opportunities employer. (8237)A

HOWARD FLOREY INSTITUTE OF EXPERIMENTAL PHYSIOLOGY AND MEDICINE
UNIVERSITY OF MELBOURNE
AUSTRALIA

DIRECTOR OF THE INSTITUTE

The Board of the Howard Florey Institute of Experimental Physiology and Medicine announces that the Institute's Foundation Director, Professor Derek Denton, will step down from that position.

The Board wishes to appoint a Director to succeed Professor Denton.

The Howard Florey is a self-governing research institute affiliated with the University of Melbourne.

The Board invites expressions of interest, before April 30, 1989, from those who may wish to be considered for appointment. It is expected that the appointment will be effective from mid-1990.

The Board reserves the right to fill the position by invitation.

Enquiries, marked confidential, initially should be made to:

Mr Kenneth B Meyer AC, DSC
President
Howard Florey Institute of Experimental Physiology and Medicine
GPO Box 2180T
Melbourne 3001
Victoria Australia

Telephone: Area Code (03) 663 3575
Facsimile: Area Code (03) 663 2975
Telex: AA35748MYR (W5787)A

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RHÔNE MÉRIEUX

Turnover : 1,2 billion FF - staff : 2000 worldwide

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Leader in veterinary research, 10 % of our turnover is invested in research.

To develop this research, we are looking for a virologist with well-based experience in general and systematic virology plus competence in biochemistry and/or immunology.

A doctor or veterinarian, he must prove his knowledge of pathology.

The job based in Lyons (France) implies a lot of travel. Knowledge of English and French desirable.

Please send letter of application, C.V. and pretentions to : **RHONE MERIEUX**
Personnel Department, P. O. Box 2006
69227 Lyon Cedex 02.

(W5797)A

TWO POST-DOCTORAL POSITIONS

are available. (1) Protein chemist with experience in immunochemistry or protein modification chemistry to investigate monoclonal antibody-photosensitizer conjugates as potential agents of cancer photochemotherapy. Familiarity with cell biology and photobiology highly desirable. (2) Biochemist/cell biologist to investigate the biochemical mechanisms of photosensitized damage to mitochondria of carcinoma cells. Experience in mitochondrial function studies desirable. Send CV to

Dr. T. HASAN,
WELLMAN LABORATORIES OF PHOTOMEDICINE,
HARVARD MEDICAL SCHOOL,
MASS GEN. HOSPITAL,
BOSTON, MA 02114.

Phone: 617/726-6996 (NW3175)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
BONE MARROW TRANSPLANT UNIT
MLSO

A qualified MLSO interested in haematology and transplantation is required for our bone marrow transplant unit. Duties include assisting with bone marrow harvests, in vitro marrow processing and laboratory monitoring of recipients after unrelated donor bone marrow transplants. Previous experience in Southern blotting would be useful, but not essential. Study for higher degrees and participation in laboratory based research projects is encouraged. There are excellent library facilities at the RPMS.

Salary: £7415 £10148-£1267 London Allowance a year.

Application forms available from the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN** quoting ref: AF23.

Closing Date: 15th February 1989 (8224)A

GEOMAR

C 3-POSITION IN PALEOCEANOGRAPHY International Distribution

GEOMAR, a Research Center for Marine Geosciences, was founded in 1987 at the Christian-Albrechts-University, Kiel, Federal Republic of Germany. Within this new Institution, the Paleoceanography Department has a position available for a **C 3-Professor in Paleoceanography** (approx. senior Associate/junior Full Professor). Applicants are expected to have demonstrated research and teaching experience in the field of paleoceanography. Apart from doing independent research work they are expected to participate in teaching marine geosciences at the Christian-Albrechts-University.

Applications from women candidates are strongly encouraged.

Applications with supporting documents and a short summary of research perspectives should be sent before **March 30, 1989** to: **the Dean, "Mathematisch-Naturwissenschaftliche Fakultät, Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40, 2300 Kiel 1" Federal Republic of Germany.** (W5778)A

ANGLO-AUSTRALIAN OBSERVATORY
Research Astronomer

The Anglo-Australian Observatory comprises the 3.9-metre Anglo Australian Telescope and the 1.2-metre UK Schmidt Telescope nea Coonabarabran, NSW, and a laboratory in the Sydney suburb o Epping, Australia. There is currently a scientific staff of 14 astrono mers (including Fellows) nominated by the United Kingdom an Australia, plus a number of visitors and associated PhD students Applications are sought for one position which will become availabl in mid 1989.

Candidates should have a strong background in physics or astro physics. The appointment will be for a fixed term of 1 to 5 years, with salary in the range Aust. $31,003-$53,099 per year, plus other allow ances, fares, etc. The successful applicant will provide assistance t visiting astronomers and contribute to the development, mainter ance and general running of the telescopes and their instrumentatior An interest in infrared instrumentation and its use would be particu larly valuable. Approximately half of the appointee's time will b spent in personal research, and there will be opportunities for co laboration with Australian and British astronomers.

Applications should include:

1. age, nationality, qualifications and experience, and date PhD.
2. research proposals for AAT time during the first year of th appointment.
   Sufficient information is required to show that proposals a competitive with applications to the time assignment panels
3. any instrument development project in which applican have a particular interest.
4. any projects involving use of the UKST, or data from th plate library.
5. starting date and length of appointment envisaged.
6. names and addresses of 3 referees, whose references th applicants should arrange to be sent directly to the Directo

Closing date 10 March 1989. All correspondence to:

The Director
Anglo-Australian Observatory
PO Box 296 Epping NSW 2121 Australia

(W5798)A

SENIOR RESEARCH TECHNICIAN

Opening for a senior research technician with extensive experience in cell ture techniques to work within a newly established Center of Molecular M cine. Good organizational and technical skills required. Send résume along v two letters of recommendation to: **Dr. Angelo M. Scanu, The University Chicago, Lipoprotein Study Unit, 5841 South Maryland Avenue, Box Chicago, IL 60637.** For information call: 312-702-1775. *The University of Chic is an Affirmative Action/Equal Opportunity Employer.* (NW3201)

Coleg Prifysgol Cymru
Aberystwyth
The University College of Wales

RESEARCH ASSOCIATE

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for a funded two-year appointment at the Post-Doctoral Research Associate level to work on the enzymology of cytokinin biosynthesis. The Research will be carried out in the Department of Biological Sciences, The University College of Wales, Aberystwyth, UK, under the direction of Dr Roger Horgan. The research will involve the isolation, characterisation and mechanistic studies of enzymes involved in cytokinin biosynthesis in both plants and micro-organisms. Preference will be given to applicants with a strong background in protein biochemistry and/or enzymatic reaction mechanisms.

The position will be on the 1A Scale with a salary maximum of £13,870 Sterling, p.a. depending upon age and experience. Applications including a c.v. and the names of two referees should be sent to **Dr R Horgan, Department of Biological Sciences, The University College of Wales, Aberystwyth, Dyfed, SY23 3DA, UK (Tel. 0970-623111, Ext 3054)** from whom further details may be obtained. Closing date for applications: 15 February 1989. (8239)A

ROYAL COLLEGE OF SURGEONS OF ENGLAND
HUNTERIAN INSTITUTE
DEPARTMENT OF BIOCHEMISTRY AND CELL BIOLOGY

POSTDOCTORAL POSITION (ASSOCIATE/FELLOW)

Applications are invited for a postdoctoral position funded by the British Heart Foundation. The work involves fundamental studies on platelet membrane biochemistry and cell biology including novel procedures for delivery of anti-thrombotic agents to intravascular lesions and thrombi. A good background in cellular biochemistry/biology is necessary. The appointment is for 2 years in the first instance. Starting salary will be £11315 (on Research and Analagous scale) inclusive of London Allowance.

For further information please telephone Professor N. Crawford (01-405 3474 Ext 3310/3311).

Applications should include a full Curriculum Vitae and the names and addresses of two academic referees, to:

Miss Vicki Ground
Personnel Office
Royal College of Surgeons of England
35-43 Lincoln's Inn Fields
London WC2A 3PN

Close date: 16 February 1989

Please quote reference: 1/89 (8241)A

CHAIR, DEPARTMENT OF PHARMACOLOGY

The University of North Dakota invites applications and nominations for a chairperson of the Pharmacology Department in its School of Medicine. Candidates should have a Ph.D. or an M.D.degree, and strong credentials in research, grantsmanship at a national level, teaching in pharmacology, as well as an aptitude for effective administration. Selection will depend on professional qualifications and the potential to provide the talent and judgement to foster an effective research and educational program in Pharmacology. Review of applications will begin on 15 February 1989 and continue until a candidate is appointed.

Applicants are to provide a letter of application, curriculum vitae, a statement of research and representative publications, and cause three letters of recommendation to be forward to: **Dr. James J. Kelleher, Chair, Pharmacology Search Committee, Office of Academic and Research Affairs, University of North Dakota, School of Medicine, 501 North Columbia Road, Grand Forks, ND 58201.**

UND is an Equal Opportunity/Affirmative Action Employer.

(NW3223)A

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
DEPARTMENT OF MICROBIOLOGY
BEECHAM LECTURER IN INFECTIOUS DISEASE
(Non-Medical)

Applications are invited for the above limited tenure position (5 years). Salary will be within the non-clinical Lecturer Grade A scale (£9,260 x 8 to £14,500) with membership of the Universities Superannuation Scheme. This is a developmental position in which the successful candidate will take part in research on molecular aspects of the pathogenicity of infectious diseases and the design of molecular probes. The position will also involve the establishment of collaborative research and teaching links within the University aimed at the formation of a Molecular Pathogenesis Group. Applicants should have a microbiology training with experience at the postgraduate and postdoctoral level in the application of molecular biological techniques to microbiological problems.

For further details contact Professor J P Arbuthnott, Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH. (Telephone 0602-421421 extension 3523).

Other particulars and a form of application may be obtained from the **Deputy Registrar and Secretary, Medical School, Queen's Medical Centre, Nottingham NG7 2UH,** to whom the completed application should be returned by the 20 February 1989. (8216)A

Statistics and Computing Statistician

A Statistician responsible for the statistical aspects of the research of the Divisions of Virology, Bacteriology and Immunobiology is required. The appointment will be initially for five years with the possibility of tenure at the end of that period.

The main duties will be the design and statistical analysis of national and international collaborate studies for WHO, and other organizations to evaluate proposed biological reference materials. There is also scope for the successful candidate to develop new statistical techniques or to collaborate in the development of computing projects, particularly in the area of expert and knowledge based systems.

Minimum qualifications are an honours degree and extensive postgraduate experience or a PhD in applied statistics. Experience in the applications of information technology in any of the following areas would also be an advantage statistics, molecular modelling, DNA and protein sequencing, databases or imaging.

The Institute is situated in a pleasant area of Hertfordshire within reasonable distance of London.

Salary will be in the range of £10,910 to £20,960 per annum inclusive, depending on qualifications and previous experience.

To apply please send a full curriculum vitae with the names and addresses of three referees to:
The Personnel Office, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG.
Telephone: 0707 54753 Ext 457.

NIBSC

Please quote reference SS/184. Closing date for applications is: 17th February 1989. (8187)A



Johann Wolfgang Goethe-Universität
Frankfurt am Main

The Department of Chemistry, Institut of physical and theoretical chemistry of the Johann-Wolfgang-Goethe-Universität invites applications for a permanent position (full professorship/C4) in

Physical Chemistry

subject to the usual university employment conditions (HUG§39a).

Applicants are expected to be able to represent the field of physical chemistry both in teaching and research.

The applicant's research field should be a topic of modern physical chemistry. It would be desirable if this field could supplement the present lines of research of the Institute, however, this is not a necessary condition.

Applicants with the necessary documents should be sent to the **Präsident der Johann-Wolfgang Goethe-Universität, Senckenberganlage 31–33, D6000 Frankfurt M.–1,** up to March 31, 1989. (W5799)A

Experimental Pathologist

Position is available for a person with an M.D. degree and trained in Anatomic and/or Clinical Pathology, and techniques of Molecular Biology. Candidate must have a demonstrated record of productivity as evidenced by prime authorship publications in critically reviewed journals and as Principal Investigator on USPHS grants or contracts. The candidate will be expected to initiate and maintain an independent, extramurally-funded research program. At least 75% of time will be available for research; and teaching Pathology to medical students and residents will be assigned.

Please send CV and a letter describing current and planned research goals, and the names of three references to:

John W. Kreider, M.D.
Division of Experimental Pathology
Department of Pathology
The Milton S. Hershey Medical Center
P.O. Box 850 Hershey, PA 17033

An Affirmative Action/Equal Opportunity Employer Women and Minorities Encouraged to Apply (NW3177)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
Genes and Cellular Controls Group

LABORATORY OF DEVELOPMENTAL BIOCHEMISTRY

Applications are invited for a three-year post-doctoral appointment to conduct research in association with Dr T J Mohun on transcriptional regulation of gene expression during early embryogenesis in Xenopus. The successful candidate will also have opportunities to collaborate with other members of the Laboratory and the Institute. The salary will be in the range of £12,520–£15,950 inclusive of London Weighting.

Applications including a curriculum vitae, list of publications, and names of two professional referees, should be sent to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA,** not later than 17th February 1989 quoting reference 0569/GDBM.

An Equal Opportunities Employer. (8219)A

MRC Medical Research Council

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
University of London

Applicants are invited for a

LECTURESHIP IN THE BIOCHEMISTRY AND/OR MOLECULAR BIOLOGY OF PHOTOSYNTHESIS

to be held within the Department of Pure and Applied Biology. The appointee will be expected to develop an independent research programme in the area of photosynthesis and should have an interest in the application of biochemical and molecular biological techniques. She/he will be closely associated with the AFRC Photosynthesis Research Group headed by Professor J. Barber and the Photochemistry Group headed by Professor Sir George Porter PRS.

Salary (inclusive of London Weighting) £10,910-£16,150.

Formal application, including curriculum vitae and the names of two referees, should be made to

Professor R.M. Anderson FRS, Department of Pure & Applied Biology, Imperial College, Prince Consort Road, London SW7 2BB by 28th February 1989.

Informal enquiries to: Professor J. Barber, tel. 01 589-5111, ext. 7433 or Fax. 01 225-1234. (8225)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF CHEMISTRY
Research Fellow

Applications are invited for the post of postdoctoral research fellow (RA1A) in the S.E.R.C. supported High Field N.M.R. Service which will be available to chemists, biochemists and biologists in U.K. universities. The successful applicant will be required to assist in the operation of the Varian VXR-600 and Bruker WH-360 N.M.R. Spectrometers under the direction of Dr. I.H. Sadler. The applicant should have practical experience in high resolution n.m.r. spectroscopy of large naturally occurring molecules including proteins and polypeptides. The appointment will be for 3 years in the first instance.

Salary scale: £9,865–£15,720.

Applications, which should include a curriculum vitae and the names of two technical referees should be sent as soon as possible to: **Dr. I.H. Sadler, Department of Chemistry, University of Edinburgh, West Nains Road, Edinburgh, EH9 3JJ.** PLEASE QUOTE REFERENCE NO. 5629 (8208)A

THE AUSTRALIAN NATIONAL UNIVERSIT

MOUNT STROMLO AND SIDIN SPRING OBSERVATORIES

POSTDOCTORAL FELLC IN ASTROPHYSICS

Applications are invited appointment as Postdoct Fellow in Astrophysics from 1989.

The Observatories (Direc Professor A. W. Rodgers) o ates seven telescopes on Stromlo and Siding Spring, in ding the new 2.3-m telescope, a wide variety of instruments optical direct imaging and pol metry, and photometry and s troscopy in the optical and in red. The main lines of research on evolutionary problems in st the interstellar medium, glob clusters, galactic structure, Magellanic Clouds, exte galaxies, radio and x-ray sou and large scale structure. A astronomers have access to Anglo Australian Telescope the aperture synthesis Austra Telescope. A PDS microph meter, VAX 11/785 and sev other VAX and MicroVAX c puters are available for data rec tion and image processing. University also has a Fuj VP100 supercomputer.

The Observatories curre comprise 20 academic staff about 80 professional techn and other support staff. There about 25 PhD students.

Closing date: *31 March 1* Ref: MS.12.1.1.

SALARY: Postdoctoral Fe Grade 1 (fixed point); A$26,6 A$30,360 p.a. APPOINTME normally two years, possibilit extension to three ye APPLICATIONS should be s mitted in duplicate to the Re trar, The Australian Natic University, GPO Box 4, Canb ACT 2601, Australia, quo reference number and inclu curriculum vitae, list of publ tions and names of at least th referees. The University rese the right not to make an appc ment or to appoint by invitatio any time. Further informatio available from the Registrar, from Appointments (359 Association of Commonwe Universities, 36 Gordon Squ London WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W5781)A

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nature

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MURDOCH UNIVERSITY
Perth, Western Australia
LECTURER IN FERMENTATION TECHNOLOGY
School of Biological and Environmental Sciences

rdoch University commenced a ad, interdisciplinary three-year gramme in Biotechnology in 7. The programme has state vernment support and a strong eraction with industry. Dr len McLoughlin, University llege, Dublin will initiate a new rse in industrial microbiology February 1989 and the new turer will be expected to further velop this course. Existing earch areas in biotechnology at rdoch University include plant ue culture and molecular biol-, algal biotechnology, mono-nal antibody production, munobiology, ethanol fermen-ion and the development of mal vaccines. The successful plicant is expected to have a ad training in microbial phys-gy and fermentation technology d preferably have had industrial perience. A record of successful earch in applied microbiology is ential. The successful applicant l be encouraged to develop nt research programmes with al industries. An enthusiasm undergraduate teaching is ential. (REF: 1080 — Post ilable from 1/10/89).

Salary: A$30,737 per annum to 40,100 per annum. (Appoint-nt will normally be made at the ver steps of the grade).

This is a tenurable appointment d conditions of appointment lude superannuation, long vice leave, outside studies pro-mme, payment of fares to Perth appointee and dependent nily, removal and settling-in owances and house purchase n arrangement.

PLICATIONS PROCEDURE APPLICATIONS, IN DUPLI-TE, quoting reference num-, including full personal parti-ars, academic record, list of blications, research interests, rent salary and names and dresses of three professional erees, should reach the Chief sonnel Officer, Murdoch Uni-sity, Murdoch, Western Aus-lia 6150 by 3 March 1989.

Applicants resident in the ited Kingdom, Europe or ica, at the time of application uld also forward ONE further y to Appointments (35961), sociation of Commonwealth iversities, 36 Gordon Square, ndon WC1H 0PF, UK.

MURDOCH UNIVERSITY IS EQUAL OPPORTUNITY PLOYER. (W5802)A

CARDIFF

PURE AND APPLIED BIOLOGY
RESEARCH ASSOCIATE

(Fixed Term — 2 Years)

EEC funded project on the transfer, spread and survival of plasmid DNA in epilithic bacteria. Research involves travel to Belgium, Spain, Portugal and Germany. PhD in Microbiology or Genetics is essential.

Salary: £9865–£15720 per annum.

For further particulars and application form please write to **the University of Wales College of Cardiff, PO Box 431, Cardiff CF1 1TA, quoting Ref 88/171.**

Closing Date: 13 February 1989.

(8228)A

ASSOCIATE OR FULL PROFESSOR
MOLECULAR BIOLOGY

A tenured position is available in the Rosenstiel Basic Medical Sciences Research Center as a joint appointment with the Graduate Department of Biochemistry. We seek candidates who are investigating basic mechanisms in molecular biology, in fields ranging from replication to gene expression. Curriculum vitae should be sent to:

Dr. David DeRosier
Rosenstiel Basic Medical Sciences Research Center
Brandeis University
P.O. Box 9110
Waltham, MA 02254–9110

The deadline for applications is 1 March 1989. *Brandeis University is an Equal Opportunity/Affirmative Action Employer.*

(NW3205)A

Harvard Medical School and Children's Hospital Boston, MA

Assistant or Associate Professor. Major emphasis is on Cell and Molecular Biology of connective and skeletal tissue, cells and noncollagenous proteins. Candidate should be experienced in isolation and characterization of proteins and genes.

Write or send résumé to: **Dr. Melvin J. Glimcher, M.D., Children's Hospital, Enders Building 11th Floor, 320 Longwood Ave., Boston, MA 02115.**

(NW3207)A

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

OXFORD LINK CENTRE
EUKARYOTIC MOLECULAR GENETICS
POSTGRADUATE RESEARCH ASSISTANT
Grade 1B BR/281

Applications are invited from suitably qualified persons for the above position in this active Centre under the direction of Drs. S. M. and A. J. Kingsman.

The research aims to study proteins that regulate viral gene expression in animal cells. Experience in virology, cell culture, or recombinant DNA techniques would be an advantage but applicants with backgrounds in other areas of Biochemistry and Cell Biology will be considered.

The position is available immediately and funding has been obtained for three years in the first instance.

Salary on the scale £8,675-£13,365 per annum.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to: **The Administrator.**

An Equal Opportunity Employer. (8209)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD,OXFORD,OX1 3QU

INSTITUTE OF CANCER RESEARCH
Sutton, Surrey
Technician

A Technician is required in the Joint Department of Physics of the Institute of Cancer Research and the Royal Marsden Hospital to assist with research aimed at improving the effectiveness of ultrasound as applied to the diagnosis and management of cancer. The post is primarily concerned with the management, processing and statistical analysis of data acquired from ultrasound images, using ORACLE relational database management software and the SAS statistical package on IBM PC and DEC VAX computers. It would thus be suitable for a new graduate in statistics, a physical science, or a related subject. An ability or an interest in learning to write computer programmes in FORTRAN is also desirable. Much of the work requires close co-operation with medical personnel. Suitable candidates must therefore be able to express themselves clearly and have good communicating skills.

The Physical Section has an active and broad-based research and teaching programme and provides a stimulating environment for someone wishing to work in the biomedical field. The post will be based at the Sutton, Surrey campus with occasional need to travel to our other branch at Fulham, London. Good sports and social facilities are on-site.

Salary in the range of £7811 to £10711.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Informal enquiries can be made to Dr. J.C. Bamber on 01-642-6011, ext. 3343.

To apply, please submit a Curriculum Vitae in duplicate with the names and addresses of two professional referees to the **Personnel Office, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL** quoting reference number 1.89.T.N.65.

(8217)A

UNIVERSITY OF MANCHESTER
North Manchester Regional Virus Laboratory
TOP GRADE VIROLOGIST (SCIENTIST)

required to head a new 'AIDS Section' to be developed in the Virology Unit sited in the New Holzel Laboratory at Booth Hall Childrens Hospital and the Medical School, University of Manchester.

The appointed person will hold a Ph.D and will have considerable experience in diagnostic clinical virology together with an ability to demonstrate achievements in independent virological research and proven skills in team management.

SALARY: £20,034 p.a.-£23,978 p.a.

Informal enquiries to Professor M. Longson, Consultant Virologist, The Virology Unit, North Manchester Regional Virus Laboratory, New Holzel Laboratory, Booth Hall Childrens Hospital, Charlestown Road, Manchester M9 2AA. Tel. 061 741 5200.

Application form and job description available from **Acute Unit Personnel, North Manchester General Hospital, Crumpsall, Manchester M8 6RB. Tel. 061 795 4567 Ext. 2144/2114 quoting Ref. A10. Closing date 10.2.89.**

AN EQUAL OPPORTUNITIES EMPLOYER (8211)A

UNIVERSITY OF OXFORD

INORGANIC CHEMISTRY LABORATORY
Appointment of Adminstrator

Applications are invited for the established post of Departmental Administrator on the Academic-related administrative Grade 4 scale, £17,630 to £20,920.

The Administrator is responsible to the Head of Department for all matters concerning the non-teaching staff, external grants and the departmental accounts, the purchase of equipment, the technical and office staff and the maintenance of the building.

Further particulars may be obtained from **Dr M.L.H. Green, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, to whom application, with a curriculum vitae and the names of two referees, should be sent by 17 February 1989.** (8229)A

The University is an Equal Opportunity Employer

ZOOLOGY
UNIVERSITY OF TORONTO

The Department of Zoology invites applications for a tenure-track position at the rank of Assistant Professor to begin 1 July, 1989, or thereafter. We are seeking an outstanding and innovative scientist who has recently received his/her Ph.D. (post-doctoral experience preferred). The successful candidate will be expected to establish a vigorous, independent research program, preferably of environmentally related vertebrate physiology and to teach at the undergraduate and graduate levels. Salary, currently between $31,500 and $53,600 for an assistant professor, will be commensurate with experience and qualifications. Applications to be submitted by 15 February, 1989, should include an up-to-date curriculum vitae, an outline of future research plans and names, addresses and telephone numbers of three referees.

If they wish, applicants may arrange with referees to send letters on their behalf. Please send documentation to **Dr. Michael Barrett, Physiologist Search Committee, Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1; (416) 978-3488.**

In accordance with the Employment Equity Policy of the University of Toronto, applications are invited from all qualified women and men. (NW3196)A

THE UNIVERSITY OF TEXAS Southwestern Medical Center AT DALLAS
Department of Biochemistry

POSTDOCTORAL FELLOW POSITIONS BIOCHEMISTRY AND GENETICS OF PROTEIN TRANSPORT

Positions are available to examine the molecular genetics of protein and ribonuclear protein transport across organelle membranes in yeast. Studies will focus on *in vitro* transport methods in conjunction with available genetic and immunological probes to define structure function relationships of the transport apparatus within the nuclear envelope and mitochondrial membranes. Send curriculum vitae or call 214-688-2120, **Dr. Michael G. Douglas, Department of Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75235-9038.** An equal opportunity employer. (NW3221)A

POSTDOCTORAL POSITION

Available immediately to study human B cell differentiation. Research in progress includes determining the usage of immunoglobulin variable region genes during ontogeny, especially of CD5 (Leu-1) positive B cells. Experience in molecular biology is desirable. Please submit a letter describing current and past research activities, c.v. and the names of two references to: **Carol Nottenburg, Ph.D., Division of Clinical Research, Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104.** FHCRC is an AA/EEO, M/F/H employer. (NW3191)A

UNIVERSITY OF GLASGOW
Department of Physics and Astronomy

Applications are invited for two post-doctoral research posts in theoretical particle physics, both tenable for two years, from 1 October 1989.

Areas of interest to the group include:

1) electro-weak interactions, physics beyond the standard model and particle physics in cosmology;
2) numerical simulations of quantum field theories.

It is intended that one appointment would be made for collaboration in areas (1) and the other for collaboration in area (2).

The posts are funded through an SERC grant and appointment would be made at the usual UK salary (Range 1A for Research and Analogous staff) corresponding to age, up to age 27 on 1 October 1989. Applicants should send their curriculum vitae and request two referees to write, before the closing date of February 17th, to **Professor R.G. Moorhouse, Department of Physics and Astronomy, Glasgow University, Glasgow G12 8QQ.** (8210)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post, commencing 1st April, 1989 or as soon as possible thereafter for a fixed term of 36 months, to study the actions and metabolism of extracellular ATP inhuman articular cartilage.

The project is funded by a grant from the Wellcome Trust to Dr. A M Caswell and Professor R G G Russell (Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School).

Applicants should have a PhD or equivalent relevant experience preferably of tissue culture techniques.

Informal enquiries may be made to Dr A M Caswell (0532) 333114.

Salary on the RA IA scale (£9,865 – £11,070) according to qualifications and relevant experience.

Application forms and further particulars may be obtained from and completed applications forwarded to the **Registrar, the University, Leeds LS2 9JT (tel 333963 – direct line), quoting reference number 83/93.** Closing date for applications 16 February 1989. (8200)A

LA TROBE UNIVERSI
Melbourne, Australia

CHAIR OF GENETICS A
HUMAN VARIATION
School of Biological Scien

Applications are invited appointment to the Chair of (etics and Human Variation i School of Biological Scie which has become vacant fol ing the resignation of the fou tion professor, Professor I Parsons.

Research and teaching activ in the Department encompa broad spectrum of interests cluding human genetics, (d graphic, anthropological health, immunogenetics cancer genetics, molecular etics); ecological and conserva genetics (plants, insects, n mals, marine invertebra evolutionary genetics (ins marsupials, plants); and molec and cell biology or eukary especially mammals. A n strength of the Department diversity of approaches to study of the phenomena heredity.

The University invites app tions from distinguished scier in any field of genetical resea The successful applicant will sess an outstanding research b ground in his or her chosen f and will be expected to der strate strong academic leade and a dedicated commitmer teaching within the Departme

The University reserves right not to make an appointi or to make an appointmen invitation.

Salary: A$63,397.

Applications should quote erence number 150-0-217 an clude a curriculum vitae and names and addresses of t referees. The application, ma 'In Confidence', should be warded to the Manager, Sta and Secretariat, La Trobe versity, Bundoora, Victoria 3 Australia, by 31 March 1 Applicants resident in the should also send a copy Appointments (35948), Ass tion of Commonwealth Univ ties, 36 Gordon Square, Lor WC1H 0PF, from whom fur particulars are available.

Equal Opportunity is Unive Policy (W5791)

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nature

Please mention when replying to these advertisemen

NTRAL BIRMINGHAM IEALTH AUTHORITY

ENIOR MOLECULAR GENETICIST

lications are invited for a or Scientist to work in the ecular Genetics Laboratory in the Regional Cytogenetics at the Birmingham Maternity ital. The Department offers atal screening for selected ogenic conditions such as cysbrosis and X-linked muscular ophy. The majority of work is ine diagnosis. However, lopment research interests ncouraged.

pplicants should have a first or nd class honours degree ther with post-graduate rience in molecular genetics. ier qualifications, eg. PhD, RC(Path) would be advantas.

lary: £11,164-£14,591 p.a. er review).

iquiries to: Dr Jessie Watt 4354). Application forms and descriptions from Personnel artment, Birmingham Mater Hospital, QEMC, Edgbaston, ingham B15 2TG (tel. 021 1377 ext 4130).

osing date: 9 February 1989.

he Authority is committed to al Opportunities in Employ-t." (8227)A

POSTDOCTORAL RAINING POSITION

st-doctoral training posin available now in basic, l-biological research on e mechanisms of biosynesis and secretion of ncreatic islet hormones. ntact:

Sumer Pek, MD,
TC3920 University of ichigan Medical Center,
Ann Arbor,
MI 48109-0354
(Phone 313 936 5032).

ual opportunity/affirmae action employer.
(NW3206)A

OSTDOCTORAL POSITION:

ailable immediately to study biochemistry and molecular logy of macromolecules olved in the adherence of bacia to corneal epithelial cell mbranes. Send curriculum ae and the names of two refer-ces to:

Dr. N. Panjwani
ufts University School of Medicine
Department of Ophthalmology
136 Harrison Avenue, Box 450
Boston, MA 02111
(NW3227)A

BRITISH POLICY IN THE ANTARCTIC

London based

...to be involved in the formulation and execution of British Policy in the Antarctic, the co-ordination of Britain's bilateral relations with other Antarctic Treaty Member States and the management of Britain's international obligations under the Antarctic Treaty. The post requires a significant amount of oveseas travel.

It is likely you hold a good honours degree in a relevant scientific subject. You should have considerable specialist knowledge of polar affairs and understanding of local practical problems in particular, conservation and environmental issues. You must be capable of establishing relations and negotiating with United Kingdom and other specialists, as well as representing Her Majesty's Government in the Antarctic. An ability to speak and read Spanish or French would be advantageous.

Starting salary will be up to £24,350 with further increments, depending on performance, up to £28,700 (salaries include £1750 London Weighting and are payable from 1 April 1989).

For further details and an application form (to be returned by 17 February 1989) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours). **Please quote ref: S(B)677.**

The Civil Service is an equal opportunity employer

FOREIGN & COMMONWEALTH OFFICE

(8251)A

ETH ZÜRICH

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ETH-Zürich

Laboratory for Biochemistry

CELL BIOLOGIST

Applications are invited for a 3 year postdoctoral position involving research on naturally occurring antibodies with binding affinities to tumour cells. The applicant must have a Ph.D. in Cell Biology or related fields and should have at least one year's experience in tumour cell line culturing with serum-free media. The candidate should have knowledge in characterizing such cells. Candidates with a background in the fields of complement and/or humoral immunology are preferred.

Interested persons are invited to submit a C.V., a list of publications, and the names of 2 persons as references to **Dr. Hans U. Lutz, Laboratory for Biochemistry, ETH-Zentrum, CH 8092 Zurich, Switzerland. For more information contact 01 256 3009 in Switzerland.** (W5795)A

THE UNIVERSITY OF CALIFORNIA IRVINE CANCER CENTER

is seeking an assistant adjunct professor of medicine. Cancer research with expertise in molecular and cell biology. Study of transformation of human melanocytes and chemoprevention. Funding for at least 2 years available. Opportunity to develop independent career. Postion available July 1, 1989.

Send letter, list of at least 3 references and curriculum vitae to: **Faculty Recruitment (Laboratory) Frank L. Meyskens, Jr., M.D., Director, UCI Cancer Center, 101 The City Drive, Building 44, Route 81, Orange, CA 92668.** Applications accepted until April 1, 1989 or until position is filled.

UCI is an EOP/AA employer. (NW3190)A

PLANT PATHOLOGIST OR ASSOCIATE PLANT PATHOLOGIST

Position available immediately at the International Rice Research Institute, Philippines, for a virologist to become part of an interdisciplinary team focusing on rice varietal improvement and integrated pest management. The person selected will conduct basic and applied research on rice viruses, virus-vector relationships, and virus disease management, and develop an innovative disease control program. Background in molecular biology preferred.

Qualifications include a Ph.D. degree with specialization in plant viruses and virus diseases. Experience in serology and electron microscopy essential. Salary and benefits will be commensurate with those of similar international agricultural research institutions. Applications will be accepted until 1 March 1989 or until position is filled.

Applicants should send curriculum vitae and the names of three or more references to: **Dr. Klaus Lampe, Director General, IRRI P.O. Box 933, 1099 Manila, Philippines.** (W5801)A

THE UNIVERSITY OF BIRMINGHAM

UNIVERSITY OF BIRMINGHAM
DIRECTOR OF BIOMEDICAL SERVICES

The University intends to appoint a Director to undertake the duties of named Veterinary Surgeon under the Animals (Scientific Procedures) 1986 Act and to manage its laboratory animal facilities. Applications are invited for this post from candidates who are conversant with laboratory animal husbandry and the organisation of a modern animal facility. An appropriate veterinary qualification is essential and managerial experience is important; however, candidates with less experience but a particular interest would not be ruled out.

The person appointed will be responsible for the management and development of animal houses and laboratory animal services in the University.

Salary will be in the Administrative range, Grade 6 (£23,570–£26,395) depending on qualifications and experience.

Further particulars may be obtained from The Director of staffing Services, to whom applications, including curriculum vitae and the names of three referees, should be sent at the following address:
University of Birmingham, PO Box 363, Birmingham B15 2TT by not later than 17 February 1989.

An Equal Opportunities Employer (8240)A

LABORATOIRE DE GENETIQUE MOLECULAIRE DES EUCARYOTES
Molecular and genetic analysis of neurogenesis

Applications are invited for a post doctoral position available immediately. Funding guaranteed for at least one year. The successful applicant will join a group whose research focuses on the genetics and molecular biology of early events in neurogenesis in Drosophila. Current projects include the study of genes involved in the segregation of neural precursors and the acquisition of positional identity by embryonic neuroblasts. The lab is part of an institute of molecular biology whose strength is the study of eukaryotic gene expression.

Experience in molecular biology is preferred. Knowledge of French not required. Please submit a full CV and letters of recommendation to: **Drs. P. Simpson and M. Bourouis — Laboratoire de Génétique Moléculaire des Eucaryotes — 11 rue Humann — 67000 STRASBOURG — FRANCE** (W5800)A

POSTDOCTORAL POSITION IN BONE MARROW TRANSPLANTATION BIOLOGY

to study the role of adhesion receptor molecules (integrins) and their potential modulation in graft versus host disease and graft rejection after allogeneic bone marrow transplantation. A Ph.D. in biochemistry, immunology, molecular biology or cell biology is preferred. Funding is for 3 years and the position will include a competitive salary and liberal benefits.

Applicants should submit their curriculum vitae along with names of three references to **Dr. H.-G. Klingemann, Terry Fox Laboratory for Hematology/Oncology, 601 West 10th Avenue, Vancouver, B.C., Canada V5Z 1L3.** (NW3226)A

ETH ZÜRICH

Postdoctoral Position
ETH-Zürich, Switzerland

available to study the molecular basis of **chromosome- and chromatin structures in yeast** with respect to gene expression, replication, and DNA repair (Thoma & Zatchej, 1988, Cell 55, Dec. 23). The position is available for 3 years starting April 1989. The salary will be US$ 38000 to 44000 (Swiss National Science Foundation) depending on experience.
The candidate should have competence in yeast genetics and/or molecular biology and/or immunological techniques. Please call or send application to: **Dr. Fritz Thoma, Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich, Switzerland. Tel: 0041-1-3773323 or 0041-1-3773352.** (W5793)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF MICROBIOLOGY
LECTURER

Applications are invited for the above post available from 1 December 1989.

Qualifications and special interests in molecular biology of herpesvirus required.

Salary on the Academic Scale for Lecturers Grade A (£9,260-£14,500) or Grade B (£15,105-£19,310) according to qualifications and relevant experience.

Informal enquiries may be made to Professor D H Watson (tel (0532) 335647).

Application forms and further particulars can be obtained from and completed applications forwarded to the **Registrar, the University, Leeds, LS2 9JT,** (tel (0532) 333963) quoting reference number 96/45. Closing date for applications 17 February 1989. (8215)A

ASSISTANT/ ASSOCIATE MEMBERS

The James N. Gamble Institute of Medical Research is seeking investigators in the Division of Enteric Viruses. Minimum requirements for successful candidates will be completion of postdoctoral studies and the ability and interest to establish or continue active research programs. Preference is for applicants specializing in viral immunology, molecular biology of enteric viruses, or animal modeling of human enteric viral diseases. No formal teaching will be required but a joint appointment at a local university is encouraged. Salaries and benefits are highly competitive and start-up funding will be available. Applications should be sent to: **R. L. Ward, Director, Division of Enteric Viruses, Gamble Institute of Medical Research, 2141 Auburn Avenue, Cincinnati, PH 45219.** *Equal Opportunity Employer.* (NW3210)A

THE HANNAH RESEAR INSTITUTE AND UNIVERSITY OF CARD

A post is available immediat the postgraduate level to j team studying the regulati adipose tissue development ferent body sites in sheep approach involves both stud vivo and in vitro, with exte use of cell culture techniques team comprises two groups t at Cardiff and the Ha Research Institute.

The post is funded for two by the AFRC University Research Group Scheme; th ary will be on the Universit (B) scale.

Further information car obtained from either Drs Ve or Flint (0292) 76013 or Dr ((0222) 874127. Applica should be submitted to The Sc ary, The Hannah Research tute, Ayr KA6 5HL, Scotlar 10 February 1989 quoting ence HRI/164. (8171

INSTITUTE O NEUROLOG
QUEEN SQUAR

1. POSTDOCTORAL RESEARCH ASSISTANT
(grade 1A, salary scale £9,865-£11,070 plus £1,65 London Allowance.

2. RESEARCH TECHNICI
(University grade 3, salary sc £6,636-£7,008 plus £1,750 London Allowance.

Candidates for the above posts required for studies of the m chondrial genome in patients defects of the respiratory c (mitochondrial myopathies). project is for three years an funded by the Muscular Dystro Group of Great Britain and North Ireland. Work will concentrate patients shown to have deletion muscle mitochondrial DNA, in ticular analysing breakpoint reg and the distribution of dele mtDNAs in muscle using in hybridization. Experience of r vant techniques is desirable for (1); training can be provided for (2).

Further particulars can be obtai from Dr A E Harding, Departm of Clinical Neurology, Institut neurology, Queen Square, Lon WC1N (tel 01 837 3611 ext 42 to whom applications (curricu vitae and the names of two refer should be sent within two week the date of this advertisem (8190)

Opportunities in Drug Discovery Research in Japan

Pfizer Central Research at Nagoya, Japan, is offering excellent career opportunities in our expanding drug discovery research programs. The laboratories located 35 km south of Nagoya city are an integral part of Pfizer Central Research worldwide and Pfizer Taito Co. Ltd., a Pfizer Inc. subsidiary. We are seeking Japanese scientists at the Ph.D. level with degrees in Biochemistry, Immunology, Pharmacology, Microbiology, and Organic Chemistry, and 1-3 years of post-doctoral or industrial experience. In addition to strong technical expertise, all positions require extensive English communication skills and computer literacy. Specific requirements are as follows:

Immunologist/Biochemist

The successful candidate will have work expertise in cytokines, cell surface receptors, and lymphocyte activation. Initially, he or she will be responsible for developing *in vitro* assays aimed at accomplishing several goals, including evaluation of the role of different mediators in the immune response. Knowledge of tissue culture techniques is essential.

Pharmacologist

A broad knowledge of, and experience with, various animal models of inflammation is essential. The successful candidate will be responsible for developing animal models of arthritis and autoimmunity. Some background in general pharmacology and cellular immunology is desirable.

Microbiologist

A strong background in biochemistry and molecular biology of micro organisms is essential. Knowledge of taxonomy is desirable.

Natural Products Chemist

The successful candidate will become a member of our discovery fermentation group. Experience in modern isolation techniques and spectral methods is essential.

Organic Chemist

Applicants should have extensive expertise in organic synthesis. Responsibilities include design and synthesis of biologically active compounds. Keen interest in the biological aspects of drug discovery is essential.

Pfizer Taito offers a competitive salary and excellent benefits, including a relocation package. Qualified applicants should submit their resumes and a brief description of their research accomplishments to **Mr. Kenji Takamatsu, Director of Personnel, Pfizer Taito Co. Ltd, P.O. Box 226, 1-1 Nishi-Shinjuku 2-Chome, Shinjuku, Tokyo 163, Japan.**

(W5784)A

Pfizer 台糖ファイザー株式会社

ファイザー名古屋中央研究所

GROUP LEADER POSITION IN MOLECULAR NEUROBIOLOGY

Friedrich Miescher-Institut Basel, Switzerland

The Friedrich Miescher-Institut, a foundation devoted to basic research in cell biology, invites applications for an independent group leader position in neurobiology. Candidates are sought who have an interest in using molecular biology techniques to study the differentiation and the regeneration of the neuronal cell in vitro and in vivo. The candidate should have at least three years postdoctoral experience in molecular aspects of cellular neurobiology. The appointee's group will join two other groups working in the field of neuronal development, led by Dr. Andrew Matus (cytoskeleton) and Dr. Denis Monard (growth factors).

The Institute has strong research programs in cellular and molecular biology and offers an outstanding scientific environment with excellent research facilities. Employment conditions for the offered position are highly competitive. The envisaged starting date is mid-1989 or later. Applications, including an outline of the candidate's proposed research project, should be submitted by February 28, 1989 to **Dr. Denis Monard, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 BASEL, Switzerland.**

(W5748)A

Biochemist/ Pharmacokineticist

Beecham Pharmaceuticals Research Division has a long established reputation for research and development of veterinary and animal health products and we are now seeking an experienced Honours graduate or post graduate scientist for our Veterinary Pharmacology Unit. The unit is concerned with evaluation of drugs for use in treatment of disease in domestic animals.

The post involves the setting up of a small group to carry out radio-labelled residue and balance studies on compounds in animals. Experience should include the design and execution of residue studies, and applicants should also be familiar with physico-chemical analytical methods for assay of drugs and metabolites.

Conditions of employment are attractive and include flexible working hours, a non-contributory pension and life assurance scheme. The laboratories are sited in rural Surrey and assistance may be given with relocation where appropriate.

To apply, write with full c.v. stating current salary, or telephone for an application form to the Site Personnel Manager, Beecham Pharmaceuticals Research Division, Walton Oaks, Dorking Road, Tadworth, Surrey KT20 7NT. Telephone 0737 364706.

Closing date for applications: 10 February 1989.

Beecham Pharmaceuticals

(8249)A

CELLULAR AND MOLECULAR PHYSIOLOGY

THE UNIVERSITY OF IOWA COLLEGE OF MEDICINE

Applications are invited to fill several positions this year at the ASSISTANT, ASSOCIATE or FULL PROFESSOR levels. We seek individuals with a strong record of research accomplishment using molecular biology, cell biology, protein chemistry or electrophysiology approaches to study mechanisms of intracellular and intercellular signalling, regulation of eukaryotic gene expression or structure and function of membrane proteins. Modern laboratory space and substantial startup funds are available. Excellent opportunities exist for participation in established interdisciplinary programs, including Cancer, Cardiovascular, Diabetes and Endocrinology Centers. Rank and salary will be based on academic and research record. Minimum requirements include Ph.D. or M.D. degree and two years of relevant postdoctoral training. Candidates should send a curriculum vitae, description of research activities, representative reprints, and request three letters of recommendation to be sent, preferably by March 15, 1989, to:

Dr. Richard Maurer
Chairman, Cell and Molecular Physiology Search Committee
Department of Physiology and Biophysics
The University of Iowa College of Medicine
Iowa City, IA 52242

The University of Iowa is an Equal Opportunity and Affirmative Action Employer
Women and Minorities are Encouraged to Apply

(NW3232)A

RESEARCH INSTITUTE OF SCRIPPS CLINIC
DEPARTMENT OF IMMUNOLOGY
POSTDOCTORAL RESEARCH FELLOW

A postdoctoral position is available immediately for an immunologist/biochemist/molecular biologist to participate in studies of endocytosis of human histocompatibility antigens. Projects include biochemical and mutagenic analyses of structural requirements for endocytosis of histocompatibility antigens, analysis of intracytoplasmic processing/degradation pathways, and effects of endocytosis on the generation of immune responses. Experience with recombinant DNA techniques and protein analysis is desired. Salary is negotiable, depending upon experience. Send curriculum vitae and three letters of reference to: **Dr. Per A. Peterson or Dr. Shaokee Wu, Department of Immunology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California 92037.**

(NW3222)A

RESEARCH OFFICER

The Jersey Wildlife Preservation Trust is re-organising its research activities and needs a Research Officer to lead this work. Applications are invited from scientists with the following qualifications.

A PhD in a biological science and proven research experience; the ability to co-ordinate research projects through effective leadership; have the ability to write precise proposals, project outlines, grant applications and reports; use statistical techniques and computer systems; and to be able to work as a part of an enthusiastic professional team including animal curators and section heads involved in Jersey and overseas.

Applications must include curriculum vitae, description of research and career goals, publications (abstracts), statement of conservation philosophy, and names and addresses of three referees. Salary will be commensurate with education and experience and is linked to University research scales. Only non-family accommodation available.

Enquiries to: **The Zoological Director, Jersey Wildlife Preservation Trust, Les Augres Manor, Jersey, Channel Islands.** Closing date: 10th February, 1989.

(8061)A

ПЕРЕВОДЧИКИ!

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You can follow the latest Soviet research in your field, and supplement your income, by translating in your home on a free-lance basis. If you have good command of English, a good knowledge of Russian, and experience or academic training in a scientific or technical discipline, you may be qualified for our translation program. Immediate openings are available in chemistry, biology, medicine, physics, mathematics, materials science, and biomedical, environmental, and chemical engineering, as well as other fields.

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(NW3233)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF MICROBIOLOGY
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a Research Associate post to work on development of new reagents for the diagnosis of tuberculosis. This post, which is funded by the World Health Organisation, is available immediately for one year. However, in line with WHO requirements, an application for renewal of funding will be made after one year. Salary (according to age and experience) on the scale IA: £9,865–£15,720.

Applications (including c.v. and the names of two referees) should be sent to either **Dr. P.W. Andrew or Dr. G.J. Boulnois, Department of Microbiology, P.O. Box 138, Medical Science Building, University Road, Leicester, LE1 9HN.** For informal enquiries call Dr. Boulnois on 0533–522944.

(8037)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
Genes and Cellular Controls Group
Short-Term Scientific Staff Position

Applications are invited for a Short-Term (three year) appointment to the Scientific Staff tenable in the Laboratory of Eukaryotic Molecular Genetics (Head: Dr P W J Rigby). The person appointed will work with Dr Rigby on a project concerned with the molecular mechanisms involved in the spatial regulation of transcription during mouse embryogenesis. The successful applicant will have had extensive experience in the application of recombinant DNA techniques to the analysis of gene expression during embryonic development.

Salary will be in the range £12,520–£15,950 inclusive of London Weighting.

Applications including a curriculum vitae, and names of two professional referees, should be sent to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA,** quoting job reference: 0001/GEKG. Closing date for this post will be 16th February 1989.

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(8238)A

MRC
Medical Research Council

NIVERSITY OF MANCHESTER

ESEARCH ASSOCIATES N SOLID STATE THEORY

plications are invited for two RC funded postdoctoral search Associateships in the partment of Physics in support the research progamme of ofessor M.A. Moore and A.J. Bray. The Research sociates would work in one or ore of the following areas: gh Tc Superconductors; Spin asses; Neural Networks; assy System Dynamics. Salary nge £9,865–£15,720 p.a. cording to age and experi- ce. Appointments for a period one year from October 1st, 89 in the first instance, but rmally extendable for a rther period of up to two years. pplications, with a curriculum tae, statement of research inter- sts and the name of two refer- es, to **Professor M.A. Moore, epartment of Physics, The Uni- ersity, Manchester M13 9PL,** as oon as possible and, in any ase, before March 4th, 1989.

he University is an equal oppor- unities employer. (8202)A

UNIVERSITY OF OXFORD NUFFIELD DEPARTMENT OF PATHOLOGY

POSTGRADUATE RESEARCH ASSISTANT

pplications are invited for the post of ostgraduate Research Assistant to vork on gene abnormalities (and their xpression) in breast cancer. The post s for 3 years and the salary is in the ange of £8,675-£13,365.

Applications, with the names of two eferees, should be sent to: **Professor I. O'D McGee, Nuffield Department of Pathology, John Radcliffe Hospital, Headington, Oxford. Tel: (0865) 817249,** from whom further particu- ars can be obtained.

The closing date for applications is: *16 Feb 1989.*

The post is funded by the Cancer Research Campaign (U.K.).

Oxford University is an equal oppor- tunity employer. (8166)A

POSTDOCTORAL POSITIONS

available beginning Spring-Summer 1989, 1-3 years tenure. (1) Bioenergetic studies on mitochondria with altered membrane lipid composition and (2) Methionine metabolism by muscle and other tissues — a new pathway. Send résumé and three letters of recommendation to: **Dr. E. Jack Davis, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46223.**

(NW3179)A

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Merck Frosst Canada Inc., the Canadian subsidiary of Merck & Co. Inc. U.S.A., is expanding the Merck Frosst Center For Therapeutic Research located in Montreal, Canada.

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Reply, in confidence, providing a curriculum vitae and list of publications to : ***The Employment Manager, Merck Frosst Canada Inc., P.O. Box 1005, Pointe-Claire, Dorval, Quebec, Canada H9R 4P8.*** (NW3228)A

MERCK FROSST CANADA INC.

POSTDOCTORAL POSITION IN VIROLOGY

Ph.D. or M.D. with a molecular virology training to study the molecular and pharmacologic aspects of herpes simplex virus reactivation. Strong molecular virology background required. Curriculum vitae and names of three references to: **Jay S. Pepose, M.D., Ph.D., Department of Ophthalmology, Box 8096, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110.**

(NW3181)A

POSTDOCTORAL POSITION

available immediately for work on molecular aspects of cytokine regulation of fibroblast proliferation, collagen synthesis, and chemotaxis. Candidates with cell or molecular biology background preferred. Send curriculum vitae, three letters of recommendation, and statement of research interests to: **Sem H. Phan Ph.D., M.D., Department of Pathology, M4204, University of Michigan Medical School, Ann Arbor, MI 48109–0602.** *The University of Michigan is a non-discriminatory Affirmative Action Employer.*

(NW3183)A

RESEARCH SCIENTISTS MAMMALIAN CELL GENE EXPRESSION

Amgen, an industry leader in applied biotechnology, has an immediate need for 2 Research Scientists to contribute to our research programs in mammalian cell gene expression.

RESEARCH SCIENTIST CELLULAR PHYSIOLOGY

Dept. #347

This position requires a Ph.D. in mammalian cellular physiology and/or somatic cell genetics as well as a minimum of 2 years' postdoctoral experience in growth regulation and differentiation of animal cells. Familiarity with culturing various primary and established cell lines as well as experience in cloning and sorting cells and selecting metabolic and drug resistant mutants are essential. Some understanding and experience in cell culture scale-up are desirable.

RESEARCH SCIENTIST GENE REGULATION

Dept. #348

Qualified candidates for this position will have a Ph.D. and a minimum of 2 years postdoctoral experience in eukaryotic post-transcriptional gene regulation, including protein processing and secretion. Familiarity with glycosylation pathways and signal transport at the molecular level is essential.

Amgen offers an exciting and challenging research environment as well as an excellent compensation and benefits package including equity offerings. Qualified applicants should send a c.v. including references, in confidence, to:

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CA 91320 (NW3188)A

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UNIVERSITY OF LIVERPOOL

CANCER RESEARCH CAMPAIGN
Department of Radiation Oncology

Applications are invited for the non-medical post of J.K. Douglas Lecturer (Special Appointment) in Molecular Biology.

The successful applicant will be expected to interact with a group of 4 scientists investigating the potential of anti-sense oligonucleotides to inhibit oncogene expression, which has just commenced a 5 year Cancer Research Campaign programme grant. In addition the applicant would be able to develop his/her own interests in the molecular biology of cancer cells.

Initial salary on the Lecturer A or B scale (£9,260–£19,310 per annum). Grade III technical support and some recurrent expenditure will be available during the first two years of the appointment to enable the appointee to become rapidly productive.

The Department of Radiation Oncology is situated at Clatterbridge Hospital between Chester and Liverpool in a busy oncology department. Being close to both North Wales and the Lake District it offers excellent recreational opportunities.

Informal enquiries may be made to Professor Hilmar Warenius or Dr. David Tidd (051-334 7605).

Applications, together with the names of three referees, should be received not later than 20th February, 1989, by the **Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX** from whom further particulars may be obtained. Quote Ref. RV/231/N.

An Equal Opportunity Employer. (8103)A

Cancer Research Campaign

POST DOCTORAL SCIENTIST

Beatson Institute for Cancer Research
Glasgow

A vacancy has arisen for a NUCLEIC ACID OR PROTEIN MOLECULAR BIOLOGIST to join an ongoing research programme funded by the Cancer Research Campaign investigating how sets of genes come to be co-expressed during erythroid cell differentiation. The position is for three years in the first instance with a salary in the range £11070-£14500. The Beatson Institute is a modern, well equipped laboratory comprising eight groups working in the fields of gene expression and tumour biology.

Applicants with relevant experience should send a complete CV to **Dr P.R. Harrison, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Glasgow G61 1BD (Tel: 041 942 9361).** (8204)A

UNIVERSITY OF TORONTO
BOTANY

The Department of Botany, University of Toronto (Scarborough Campus) invites applications for a tenure-stream position (Assistant Professor level) in cell biology or molecular genetics. Preference will be given to individuals familiar with recombinant DNA technology. The successful candidate will be expected to participate in undergraduate and graduate teaching and to develop a strong research program. Applicants should, by March 31, 1989, submit a curriculum vitae, including a statement of research interests, and have at least three letters of reference sent to **J.C. Ritchie, Acting Chair, Life Sciences Division, Scarborough Campus, University of Toronto, 1265 Military Trail, Scarborough, Ontario, Canada, M1C 1A4.** The University encourages both men and women to apply. In accordance with Canadian Immigration requirements priority will be given to Canadian citizens and permanent residents (landed immigrants) of Canada.

(NW3225)A

PEPTIDE CHEMIST/ MOLECULAR BIOLOGIST

The Division of Endocrinology Research Laboratories at the Rhode Island Hospital seeks a Research Associate with experience in peptide chemistry and/or molecular biology to study proTRH processing and TRH biosynthesis in an NIH-supported research program. Applications with the names of three references to **Ivor Jackson, M.D., Director, Division of Endocrinology, Brown University/Rhode Island Hospital, 593 Eddy Street, Providence, RI 02903.** An equal opportunity/affirmative action employer.

(NW3176)A

ASSISTANT PROFESSOR, MOLECULAR BIOLOGY/ PEPTIDE HORMONES, McGILL UNIVERSITY

The Department of Medicine, Royal Victoria Hospital has a faculty position available immediately for a Ph.D. to work on the molecular biology of somatostatin and somatostatin receptors. Experience in cloning, expression, structure-function relationships essential. Excellent academic environment and opportunity for advancement. Send curriculum vitae and three letters of reference to

Dr. Y.C. Patel, Room M3-15, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec H3A 1A1, CANADA.

(NW3198)A

A. POSTDOCTORAL/ B. RESEARCH ASSOCIATE:

Immediate openings: Qualifications: Training in Biochemistry or related discipline; recent Ph.D for (A) and with 2-3 years postdoctoral experience for (B). Research involves metabolism and functions of carnitine. Experience with immunological and molecular biological approaches would be helpful. Salary: for position (A) $20,655., for position (B) $24,135. Send C.V. and letters of recommendation to Dr. Shri V. Pande, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7. (NW3197)A

School for Field Studie

School for Field Studies seek Sr. & Assoc. faculty for summe programs: Ethnobot./Ecuado Landscape Ecol./Colorado; Tr pical Ecol./Costa Rica; Acid Rai Adirondacks; Wildlife Mngt Kenya; Cult. Ecol./Himalaya Bald Eagles/Alaska; Coral Re Ecol. & Marine Pks. Mngt./Virgi Isles; Monkseals/Greece; Spinn Dolphins/Hawaii; Humpbac Whale Ecol./N. Atlantic; Dolphin Alabama. Ph.D. (MS. for Asso Fac.), excel. teaching skills, fiel resch. exp., enthus. for experier tial ed. Send detailed letter, c and refs. to the **Dean, SFS, 1 Broadway, Beverly, MA 0191 (508) 927-7777.** (NW3203)A

The **Biocenter of the Universit of Basel** has an open positio for a

PROTEIN CHEMIST

who will set up and run a servic facility including protein sequen cing, peptide synthesis an amino acid analysis in collabora tion with groups in the Biocen ter. The position is available immediately and is provide with a competitive salary. It ma be converted into a permanen appointment.

Applications should be sen to the

Erziehungsdepartment Basel-Stadt,
Personalsekretariat, Postfach, 4001 Basel

and should include a curriculun vitae, list of publications and th names of three referees.

(W5786)A

POSTDOCTORAL POSITION

Available from April 1989 t work on regulation of expres sion and function of the *c-mos* proto-oncogene. Applicant should have a molecular bio logy training and an interest ir mouse embyogenesis. Send curriculum vitae and names o three referees to:— **Dr. F Propst, Ludwig Institute fo Cancer Research, St. Mary's Hospital Medical School Norfork Place, Paddington London W2 1PG (Telephone 01 724 5522).** (8130)A

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You should have a degree or PhD in chemistry, biochemistry or a related discipline, and several years' experience in drug metabolism gained in the pharmaceutical industry or a similar environment. You should also have supervisory experience.

Experience in the use of various chromatographic and radiochemical techniques is expected and the possession of a Home Office Licence and experience of working to GLP standards would be an advantage.

Your starting salary will reflect your experience, and additional benefits will include a non-contributory pension scheme, profit-related bonuses and active sports and social club. Generous relocation assistance will be given where appropriate.

If you wish to take this opportunity of joining the market leaders, please write or telephone for an application form to Mr M J Linnane, Personnel Officer, Glaxo Group Research Limited, Park Road, Ware, Herts SG12 0DP. Tel: Ware (0920) 3993 ext 2293.

Glaxo Group Research

(8250)A

POSTDOCTORAL POSITIONS

Postdoctoral positions are available in cell, molecular and structural biology of membrane (D.W. Hamilton) and cytoskeletal systems (R.W. Linck). Membrane projects include: biogenesis during spermatogenesis, glycosylation, formation of the acrosomal, head and flagellar membranes, subsequent membrane maturation within the epididymis, and the function of membrane proteins. Cytoskeleton projects include: molecular structure and assembly of microtubules, gene structure and expression of microtubule proteins in development, and in vivo function of microtubules in cilia/flagella, centrioles/centrosomes and mitosis/meiosis. Experience is required in preferably more than one of the following: cytology, protein chemistry, immunochemistry, EM, diffraction methods, and/or recombinant DNA methods. Send curriculum vitae and three letters of reference to: **Dr. D.W. Hamilton or Dr. R.W. Linck, Department of Cell Biology and Neuroanatomy, University of Minnesota, 4-135 Jackson Hall, 321 Church Street, S.E., Minneapolis, MN 55455.** (NW3218)A

Director, Genetic Diagnostic Laboratory:

A position available immediately for a Ph.D. with experience in molecular biology/biochemical genetics to direct a genetic diagnostic laboratory. There is ample opportunity for independent research and faculty appointment at Duke University Medical Center (rank dependent on experience). The candidate will join an established and growing, comprehensive academic genetic and metabolic program with expertise in clinical genetics, molecular and biochemical genetics associated with a major mass spectrometry facility. Duke University is an Equal Opportunity/Affirmative Action Employer. Send curriculum vitae and three references to:

Y.T. Chen, M.D., Ph.D.
Associate Professor of Pediatrics
Division of Genetics and Metabolism
Department of Pediatrics
P.O. Box 3028
Duke University Medical Center
Durham, North Carolina 27710 (NW3161)A

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2 POSTDOCTORAL POSITIONS IN MOLECULAR CELL BIOLOGY

Two positions are available immediately to study the molecular mechanisms of cell and tissue interactions which regulate cell differentiation in the embryonic visual system. Emphases are on the role of plasma membrane molecules and molecular genetics. Salary commensurate with experience.

Send C.V. and three letters of recommendation to:

Dr. Paul J. Linser
Whitney Laboratory
University of Florida
9505 Ocean Shore Blvd.
St. Augustine, FL 32086

An affirmative action equal opportunity employer. (NW3146)A

POSTDOCTORAL FELLOW

Postdoctoral positions are available in a molecular biology-electrophysiology laboratory for research on the structure-function relationship of brain and cardiac sodium, potassium and calcium channels. Site-directed mutagenesis and hybrid chimeras are used to construct channel mutants for expression in Xenopus oocytes and stably transfected cell lines are being established. Channel technology would be helpful. Interested applicants should send resume and addresses for three letters of reference to:

Rolf H. Joho, Ph.D., Assistant Professor or Arthur M. Brown, M.D., Ph.D., Professor and Chairman, Department of physiology and Molecular Biophysics, Baylor College of Medicine, One Baylor Plaza, Texas Medical Center, Houston, Texas 77030.

We are an equal opportunity employer. (NW3213)A

SALISBURY HEALTH AUTHORITY
WESSEX REGIONAL CYTOGENETICS UNIT
Salisbury General Hospital — Salisbury — Wilts

SENIOR SCIENTIST

A Senior Scientist is sought to join an expanding Molecular Genetics Section primarily involved in the diagnosis of a variety of inherited disorders. Experience in molecular techniques is essential. Applications are invited from:

1) Post Graduates with a first or second class honours degree and a minimum of four years experience in a related field or
2) Post Doctoral individuals with a degree in molecular biology and relevant experience.

Salary: £11,164-£14,591 (award pending).

Enquiries to Dr Patricia A Jacobs or Dr John Harvey. Telephone 0722 336212, Extension 669.

FOR AN APPLICATION FORM AND JOB DESCRIPTION PLEASE WRITE TO THE PERSONNEL DEPARTMENT, ODSTOCK HOSPITAL, SALISBURY, WILTSHIRE. SP2 8BJ.

CLOSING DATE: 16 February 1989. (8248)A

MOLECULAR NEUROBIOLOGY

THE UNIVERSITY OF IOWA COLLEGE OF MEDICINE

Applications are requested from individuals with active research programs using approaches of molecular biology or membrane biophysics to address problems broadly related to mechanisms of signal transduction and synaptic communication for faculty positions at the ASSISTANT, ASSOCIATE, or FULL PROFESSOR levels. Modern laboratory space and substantial startup funds are available. Excellent opportunities exist for interaction with faculty associated with formal Neuroscience and Molecular Biology Ph.D. programs. Rank and salary will be based on academic and research record. Minimum requirements include Ph.D. or M.D. degree and two years of relevant postdoctoral training. Candidates should send a curriculum vitae, description of research activities, representative reprints, and request three letters of recommendation to be sent, preferably by March 15, 1989, to:

Mary Boyle, Ph.D.
Molecular Neurobiology Search Committee
Department of Physiology and Biophysics
The University of Iowa College of Medicine
Iowa City, IA 52242

The University of Iowa is an Equal Opportunity and Affirmative Action Employer
Women and Minorities are Encouraged to Apply

(NW3234)A

POSTDOCTORAL RESEARCH ASSOCIATE

Position available immediately at Stanford University Medical Center to investigate mechanisms of acute lung injury and strategies for prevention. Lab investigates lung injury utilizing small animal models, cell culture techniques, neutrophil signal transduction, pathway biochemistry, patch-clamping, and receptor interaction studies. This position will have some supervisory responsibility. Approximate annual salary is 32K. Please send curriculum vitae, names of three references and brief description of interests, experience and mastered investigative techniques to: **Thomas A. Raffin, M.D., Acting Chief, Division of Respiratory Medicine, Room C-356, Stanford, CA 94305.**

(NW3133)A

agc

RESEARCH MANAGER

CAMBRIDGE **Competitive salary + Car**

Agricultural Genetics Company Limited (AGC), a leading biotechnology specialist, was formed in 1983 to develop and market products and licensing opportunities in plant biotechnology. Products derived from AGC supported research have resulted in direct market launches and commercial partnerships worldwide. The Company currently funds over 100 research scientists in more than 50 specific teams in AFRC Institutes and British Universities and is structured around three strategic business units.

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A vacancy has arisen in the corporate R & D research management team based at the Company's central office on the Cambridge Science Park.

The position of RESEARCH MANAGER will involve the identification, placement and management of research projects with commercial potential in research institutes and universities.

Applications are invited from candidates who are qualified to PhD level in one of the biological sciences with several years relevant research experience.

The company offers excellent conditions of employment including competitive salary, company car, company pension and health insurance schemes.

Please send your full CV and details of two referees to:

Dr Paul Boseley, Director of Research and Development, Agricultural Genetics Company Limited, 154 Science Park, Milton Road, Cambridge CB4 4GG

Closing Date for applications February 9th 1989

(8252)

POSTDOCTORAL FELLOW

A postdoctoral position is open for study of the biochemistry and cellular functions of membrane structural proteins in mammalian cells. Current projects include study of the proteins interacting with the spectrin-based membrane skeleton of brain and epithelial cells, and the role of spectrin and ankyrin in localization of membrane proteins at specialized cell domains such as nodes of Ranvier and basolateral domains of epithelial cells. Qualifications: Ph.D. with background and publications in either molecular biology, biochemistry, or cell biology. Please address applications to: **Vann Bennett, Howard Hughes Medical Institute, Department of Biochemistry, Duke University Medical Center, Box 3892, Durham, NC 27710.**

(NW3231)A

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RESEARCH ASSOCIATE

in molecular and biochemical basis of drug action with emphasis on AIDS Therapeutics. Ph.D. with experience in enzymology or molecular biological approaches highly desirable. Salaries are commensurate with experience and begin at $22,000. Start date is flexible.

Send curriculum vitae and names of three references to: Arnold Fridland, Ph.D., Department of Pharmacology, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38101. (NW3093)A

Ph.D. IN IMMUNOLOGY/ VIROLOGY/MOLECULAR BIOLOGY

needed by young company in Boston area to work on development of novel products for HIV testing, antibody-based research/ diagnostic tests, and nucleic acid hybridization. Focus will be on new laboratory instruments. Candidate should have experience with protein/DNA electrophoresis, western and southern blotting, and other molecular biology techniques. Please send curriculum vitae to:

Carol Watkins, Immunetics Inc., 380 Green Street, Cambridge, MA 02139.

(NW3184)A

POSTDOCTORAL POSITION

available immediately to characterize the biochemical and functional properties of extracellular/ matrix proteins involved in the expression of the transformed phenotype. Background in biochemistry, cell biology, molecular biology or immunology desirable. Send curriculum vitae and three letters of reference to:

Dr. Susan P. Hawkes, Department of Pharmacy, S-926, University of California, San Francisco, CA 94143-0446.

(NW3215)A

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when replying to these advertisements

UNIVERSITY OF OXFORD

OXFORD CENTRE FOR MOLECULAR SCIENCES
Director: Professor J E Baldwin FRS

A new interdisciplinary research centre involving 10 departments at the University of Oxford has been established in the field of molecular and protein sciences with funding from SERC, MRC and industry. Research projects are concentrated in 6 areas: Blood clotting and fibrinolysis, Immunology; Signal transduction; Viruses; Enzymes of secondary metabolism; Protein folding and specificity. These projects will have access to the core research facilities of the Centre (crystallography, NMR spectroscopy, computation and graphics, glycobiology, organic chemistry) all of which are equipped to a very high level.

GRADUATE STUDENTSHIPS

A number of graduate studentships are available and we invite prospective graduate students to write for further information, giving some indication of their research interests.

POSTDOCTORAL FELLOWSHIPS

We invite those at a postdoctoral stage to write for further information, giving some indication of their research interests. In particular we have, at present, postdoctoral vacancies in the following areas:

FREE ENERGY METHODS
Computational chemist with a background in statistical mechanics or quantum chemistry to apply the free energy perturbation technique to protein-protein interactions and enzyme-inhibitor complexes.
Dr W G Richards, Dr G Lowe

ENZYME-BOUND INTERMEDIATES
Methods for investigating enzyme-bound intermediates by means of solid state NMR will be applied in mechanistic studies of β-lactamases. Experience of protein chemistry and/or NMR spectroscopy would be an advantage.
Dr S G Waley, Dr C M Dobson, Prof J E Baldwin, Dr A E Derome

STRUCTURE OF RECEPTOR FRAGMENTS
To investigate receptors associated with trans-membrane signalling. High resolution NMR, computation and molecular modelling will be used to determine structures of receptor fragments produced by peptide synthesis. Experience of structure determination by NMR and/or modelling would be an advantage.
Professor R J P Williams, Dr I D Campbell

NMR METHODS MULTIDOMAIN PROTEINS
NMR spectroscopist required to develop methods for the study of the structures, dynamics and folding properties of multidomain proteins, particularly those associated with blood clotting and fibrinolysis.
Dr C M Dobson, Dr C C F Blake, Dr P J Hore, Dr R A G Smith (Beechams)

COAGULATION FACTORS AND DOMAINS
Protein chemist with experience in protein purification and chemical modification of proteins required for work on structure & function of the various domains in coagulation factors and the proteins involved in fibrinolysis.
Dr C C F Blake, Dr I D Campbell, Prof R J P Williams, Prof G G Brownlee, Dr M P Esnouf

BLOOD COMPLEMENT SYSTEM
Protein chemist with strong molecular background required to work on synthesis of small structural domains found in blood complement system proteins, using yeast expression systems. Expressed domains will be used for analysis of tertiary sturcture by NMR & for functional studies.
Dr K B M Reid, Dr I D Campbell, Dr R B Sim

VIRAL AND SUBVIRAL STRUCTURES
Protein chemist and/or X-ray crystallographer required to work on crystallisation and structural analysis of various viruses and viral proteins using X-ray Laue diffraction.
Prof D H L Bishop, Dr J Hadju

INFLUENZA VIRUS NUCLEOPROTEIN
Protein chemist and/or crystallographer required for purification and X-ray structural analysis of influenza virus nucleoprotein (A and B subtype) to be purified from recombinant DNA sources.
Prof G G Brownlee, Dr D I Stuart

ANTIBIOTIC BIOSYNTHETIC ENZYMES
β-lactam biosynthetic enzymes cloned and expressed in *E. coli* will be prepared with isotopically labelled constitutent amino acids for structural and mechanistic NMR studies. This research will involve molecular biology and protein chemistry; experience in either area would be advantageous.
Prof J E Baldwin, Dr C M Dobson, Dr A D Derome, Dr A J Pratt

The Centre is funded for 6 years in the first instance, with all postdoctoral positions being tenable for 2 years initially, with the possibility of extension, on the scale RSAA1A £9865–£12760 (according to age and experience)

OMSC BORE STAFF POST NMR Spectroscopy

Research assistant required to join a small team who maintain and develop NMR spectrometers as well as work with a larger team who work on the structure and function of a variety of proteins. Excellent facilities include a 600 MHz, a 360 MHz and 2 500 MHz spectrometers, a micro vax and a cluster of SUN 4 computers. Some experience of sophisticated modern hardware is required, preferably with NMR.

The post is SERC funded for 5 years in the first instance on the scale RS1A £9865–£12760 according to age and experience.

TECHNICAL POSTS Peptide synthesis

OMSC has recently established a peptide synthesis facility which contains state of the art synthesis and purification equipment. A technician is required to operate and maintain an Applied Biosystems synthesiser, assisting members of the Centre to carry out peptide synthesis and to supply purified peptides to Centre members. Candidates with an interest in peptides and who have experience in some area of peptide or protein chemistry are preferred. HNC or equivalent is desirable but experience is of equal importance.

Microbiology

A technician is required to assist in the Microbiology facility. Duties will include preparation and maintenance of bulk enzyme stocks, enzyme purification, fermentation of genetically engineered bacteria, organisation and maintenance of laboratory equipment. Also making, dispensing and sterlising bacterial growth media. Experience of microbiological sterile technique is preferable and/or enzymology/biochemistry experience.

Both posts are SERC funded for 5 years in the first instance and on the scale T5 (£8,088–£9,549)

Applications for all posts should be made in writing, enclosing a full CV and the names of 2 referees and sent to **Sarah Hargreaves, Oxford Centre for Molecular Sciences, c/o Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY (0865 275654)**. Closing date for applications: 24 February 1989. (8236)A

PLANT MOLECULAR BIOLOGY/BIOCHEMISTRY ASSISTANT, ASSOCIATE, FULL PROFESSOR

The Department of Biology at The University of Michigan seeks applications for 2 positions. Both positions are on the tenure track, and one position is authorized at the level of Associate or Full Professor. We seek applications from individuals who utilize the techniques of plant molecular biology/biochemistry in attacking significant problems related to growth, development or metabolism in plants or algae. The department is now in a period of facilities renewal, and new faculty will be housed in modern laboratories. In addition, the Matthaei Botanical Gardens, a unit of Biology, provides excellent facilities for the growth of experimental material. Biology, in cooperation with the Department of Chemistry, has facilities for oligonucleotide synthesis and molecular graphics; peptide sequencing facilities are close at hand in the Medical School. The successful candidates will be expected to conduct active research programs and participate in the graduate and undergraduate teaching programs of the department. Applicants for a position at the Assistant Professor level should send a current c.v. and brief statement of future research plans. Applicants should also request letters of recommendation from at least 3 referees. Applicants for a tenured position should submit a c.v., statement of future research plans, and the names of 4 references. Candidates for the senior position will be contacted before references are sought, and all communications will be handled in strictest confidence. Application materials for both positions should be addressed to:

Chair, Plant Molecular Biology Search Committee
Department of Biology
The University of Michigan
Ann Arbor, MI 48109-1048

Evaluation of applications will begin on March 15, 1989 and continue until these positions are filled.

The University of Michigan is an Affirmative Action/Equal Opportunity Employer. (NW3237)A

Scientists

Paravax, Inc., an innovative new company focusing on the development o vaccines against parasites, has challenging opportunities for research scientist at all levels. Applicants should have training in one of the following areas:

- cDNA cloning methodology
- heterologous gene expression systems including living recombinant vectors
- mammalian or invertebrate cell culture.

A background in the immunology or molecular biology of parasitic disease or i veterinary vaccine development is also desirable. For consideration, pleas send résumé and names of references to:

Scientific Director, Paravax, Inc.,
3181 Porter Drive, Palo Alto, CA 94304 (NW3245)A

Paravax, Inc.

MOLECULAR BIOLOGY

Applications for a tenure-track position at the advanced assistant professor level are invited from scientists with experience in molecular biology. We seek applicants with sufficient experience to merit immediate consideration for promotion to associate professor with tenure. The appointee will be required to maintain an active research program and to teach a course and laboratory in molecular biology. Start-up funds and laboratory space are available for establishing a vigorous research program.

Oakland University is a state-supported institution situated on a 1,400-acre campus 25 miles north of Detroit. The Department of Biological Sciences is a well-equipped, research-oriented department in the College of Arts and Sciences.

Applicants should submit curriculum vitae, a description of their research plans, key reprints, and names of three references before 15 February 1989:

Dr. V.K. Moudgil, Chair, Search Committee
Department of Biological Sciences
Oakland University
Rochester, MI 48309-4401

Affirmative Action/Equal Opportunity Employer.

(NW3236)A

RESEARCH SCIENTIST PHARMACOLOGY

Amgen is a dynamic, growing organization producing human therapeutic products through recombinant DNA technology. Our leadership in the development of Erythropoietin and Granulocyte Colony Stimulating Factor has placed us at the leading edge of the biotechnology industry. Our continued growth has created an immediate opportunity for a Research Scientist in our Pharmacology Group.

Applicants must possess a Ph.D. in Pharmacology or a related discipline with a strong background in whole animal research and experience in one or more of the following areas: inflammation, wound repair, immunopharmacology, hematology and oncology. Proficiency in statistical analysis of data is essential, and postdoctoral training is highly desirable.

Amgen offers a competitive compensation and benefits package, including equity participation, as well as a challenging and stimulating research environment. Our location one hour northwest of Los Angeles offers the best in Southern California living. Qualified applicants should direct a resume and a list of 3 references, in confidence, to:

Recruitment, Dept. #372
AMGEN
1900 Oak Terrace Lane
Thousand Oaks
CA 91320 (NW3244)A

Equal Opportunity Employer

AMGEN

CLINICAL PROJECT MANAGERS

The Institute of Clinical Pharmacology (Irl.) Ltd., a world leader in drug research, is a fast growing, dynamic, Irish company with subsidiaries in the United States, U.K., Switzerland and Japan. Our clients include many major pharmaceutical companies throughout the world. Due to continuing expansion we now require Clinical Project Managers.

Ideal candidates should have medical or science qualifications with proven ability to establish and conduct clinical trails to meet with international regulatory requirements. The successful applicant will be based in Dublin but must be prepared to travel. Language skills and computer literacy would be an advantage.

Curriculum Vitae, which will be treated in the strictest confidence, should be submitted to:

ICP

Director of Personnel,
Institute of Clinical Pharmacology,
Sir Patrick Dun's Hospital,
Dublin 2.

ICP Joint venture HCA (8253)A

POSTDOCTORAL POSITION

ailable immediately to join a research am working on molecular approaches study viral diseases. Experience in ochemistry, molecular biology or cell ology required. Salary commensurate ith qualifications and experience. cellent fringe benefits. Send curric- um vitae, a brief summary of research terests, and three letters of reference **: Dr. Beatriz G.T. Pogo, Box 1061, The ount Sinai School of Medicine, epartment of Neoplastic Diseases, One ustave L. Levy Place, New York, Y 10029-6574.**

An Equal Opportunity Employer.

(NW3239)A

PROTEIN CRYSTALLOGRAPHY AND STRUCTURAL CELL BIOLOGY

Applications are invited for a **post-doctoral position** involving application of these disciplines in an on-going x-ray crystallographic study of a number of actin-binding proteins, including profilin, involved in the cytoskeleton and cell motility. The position is sponsored jointly by Drs. Thomas D. Pollard and Eaton Lattman at the Johns Hopkins University School of Medicine. Membership in the newly established Institute for Biophysical Research on Macromolecular Assemblies may be possible. Experience in at least one of these areas is preferred, but persons with experience in physics or protein chemistry and an interest in moving into biophysics are also encouraged to apply. The position is available in the spring for a period of 2 to 3 years. Salary $18,000-$20,000 depending on experience. Please contact either **Dr. Thomas D. Pollard, Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, MD 21205-2185, (telephone 301-955-5664), or Dr. Eaton Lattman, Department of Biophysics, Johns Hopkins Medical School, Baltimore, MD 21205-2185, (telephone 301-955-1210).** Johns Hopkins is an equal opportunity, affirmative-action employer. Applications from minorities or women are particularly encouraged.

(NW3241)A

University of Southern California

GRADUATE RESEARCH training positions in THE MARINE BIOLOGY RESEARCH SECTION of the Department of Biological Sciences. Students will receive Ph.D. degrees through the Department of Biological Sciences. Stipends and tuition waivers available for eligible candidates. Research areas include: at the organismal level, the biochemistry, physiology and evolution of marine bacteria, algae and invertebrates; at the ecosystem level, bacterial and plankton productivity, and community structure in polar, tropical and temperate ecosystems. Contact: **Dr. C.W. Sullivan, Marine Biology Research Section, Dept. of Biological Sciences, U.S.C., Los Angeles, CA 90089-0371.** (NW3246)A

READVERTISEMENT
BRUNEL UNIVERSITY
DEPARTMENT OF BIOLOGY AND BIOCHEMISTRY
READERSHIP IN CELL BIOLOGY

Applications are invited for a Readership in the Department of Biology and Biochemistry (Head of Department: Professor T.F. Slater) to work in the general area of cell biology/pathology.

Applicants should be established scientists with outstanding research ability. The successful candidate will play a major role in strengthening and expanding the existing Cell and Molecular Biology research group within the department. The present interests of this group include macrophage cell biology, comparative reproduction, cloning of catabolic genes, and parasite immunology. The department also has very well established research groups working on free radical biochemistry, and biochemical mechanisms of cell injury, and is well equipped for research in these areas.

It is hoped the successful candidate will be able to start on 1 July 1989 or as soon as possible thereafter. The salary will be within the Reader scale, (21,720-£24,360 inclusive of London Allowance, with USS benefits).

Further information is available from Professor T.F. Slater, and applications should be returned to the **Personnel Secretary, Brunel University, Uxbridge, UB8 3PH** by 15 March 1989. (8207)D

Post-doctoral positions

1) studying the multiple roles f EGF receptors during mouse levelopment. Techniques in- lude embryo dissection, cell ulture, recombinant DNA onstruction and transfection, mmunohistology and receptor neasurements. (2) working on he development of epithelial ayers using embryonal carci- oma cells and cell-cell and ell-matrix interactions. Send V and names of 3 referees to: **lr. Eileen Adamson, La Jolla ancer Research Foundation, 0901 N. Torrey Pines Road, La olla, CA 92037 (619) 455-6480.** OE/AA. (NW3167)A

POSTDOCTORAL POSITIONS

available beginning Spring-Summer 1989, 1-3 years tenure. (1) Bioenergetic studies on mitochondria with altered membrane lipid composition and (2) Methionine metabolism by muscle and other tissues — a new pathway. Send résumé and three letters of recommendation to: **Dr. E. Jack Davis, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46223.**

(NW3240)A

Applications are invited for a **College Lectureship** post in each of the following departments:

BIOCHEMISTRY
FOOD CHEMISTRY
MATHEMATICS
ZOOLOGY

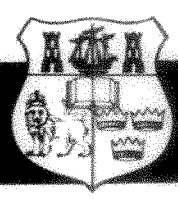
Preference will be given to candidates for the post (a) in **Biochemistry** who have research experience in advanced protein chemistry or advanced enzymology, and (b) in **Zoology** who have expertise in molluscan biology and culture.

Salary scale: IR£12,488 - IR£17,063
Bar IR£18,956 - IR£24,968 p.a.

Application forms and further details may be obtained from the undersigned. Tel.010-353-21-276871, ext. 2364. Latest date for receipt of completed applications is **Friday, 24 February, 1989**

M.F. Kelleher
Secretary

Coláiste na hOllscoile Corcaigh
University College Cork



nature

the widest international selection of jobs in science — EVERY WEEK

CARDIOVASCULAR RESEARCH TRAINING AT THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY PROGRAM OF EXCELLENCE IN MOLECULAR BIOLOGY

Postdoctoral Research Training Fellowships in the Molecular Biology of the Blood Vessel Wall are available for candidates with a M.D., or Ph.D. degree at a NIH-HLB, sponsored Program of Excellence in Molecular Biology of the Cardiovascular System. The twin goals of this Program are:

1) to develop an environment in which the most effective strategies of molecular biology will be employed to expand our knowledge of the function and malfunction of the cardiovascular system.

2) to train young investigators who will apply basic science approaches to the continued resolution of major unanswered questions in this area.

Our training program is designed to foster extensive interactions between the trainees and senior faculty at MIT as well as at a variety of Universities and Medical Institutions and to prepare these scientists for independent research careers.

Research opportunities are available in the laboratories of one or more of the following participating training faculty:

Robert Rosenberg	(Endothelial Cell Receptors)	Harvey Lodish	(Glucose Transporters)
Monty Krieger	(Genetic Analysis of Acetyl-LDL Receptors & Foam Cells)	Rudolf Jaenish	(Collagen and Cardiovascular Structure/Function)
David Housman	(Genetic Predisposition to Cardiovascular Disease)	Richard Mulligan	(Genetic Therapy of Hyperlipidemia)
Richard Hynes	(Fibronectin and Cardiovascular structure/function)	Earl Ruley	(Oncogene Induced Lipogenesis)

Interested candidates should send a resume indicating any preference in possible mentors to:

Robert D. Rosenberg, Program Director, Program of Excellence in Molecular Biology of the Cardiovascular System
Massachusetts Institute of Technology, Department of Biology
77 Massachusetts Ave., Cambridge, MA 02139. Telephone 617 253 8804

M.I.T. is an affirmative action/Equal Opportunity Employer (NW2823)E

UNIVERSITY OF GLASGOW DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL FELLOWSHIP

Applications are invited for an SERC Postdoctoral Fellowship, for which funding is available for 2 years, (1a scale, starting salary according to age and experience) to investigate the origins, structure and function of protein X, a recently-discovered component of eukaryotic pyruvate dehydrogenase multienzyme complex. The project will involve comparative sequence analysis of the human protein X and lipoate acetyltransferase (E2) genes to determine the relationship between these two polypeptides. Isolated gene sequences will be employed in studies on the targeting, import and intramitochondrial assembly of the high M_r core structure of the complex.

Qualified candidates should send a detailed C.V. **as soon as possible to Dr. J.G. Lindsay, Department of Biochemistry, University of Glasgow, Glasgow, G12 8QQ (telephone 041- 339 8855 Ext. 4720) from whom further details may be obtained.** Some experience in molecular, biological, immunological and protein-chemical techniques is preferable although not essential. (8175)E

THE NORMAN AND SAIE LEE RESEARCH CENTRE
LABORATORY OF NEUROBIOLOGY
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
POSTDOCTORAL FELLOWSHIP

A postdoctoral physiologist/anatomist is required to join a team investigating central nervous system plasticity using transplantation techniques. The fellow will join a multidisciplinary group of scientists headed by Dr Geoffrey Raisman and will be directly involved in studies of the anatomy and electrophysiology of transplanted neurons. Substantial experience with intracellular techniques is essential. The appointment is for a period of three years and will involve direct collaboration with Dr Michael Frotzcher, University of Frankfurt.

Salary will be in the range £12,520–£15,950 inclusive of London Weighting.

Applications including a curriculum vitae, list of publications, and names of two professional referees, should be sent to **Mr C R Russell Administrative Manager, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA.** Closing date for this post will be 9th February 1989. Please quote reference ISRT/5.

An Equal Opportunities Employer. (8212)E

MRC
Medical Research Council

INTER-UNIVERSITY CENTRE FOR ASTRONOM AND ASTROPHYSICS (IUCAA)
POST-DOCTORA POSITIONS

IUCAA invites applicatio for post doctoral fellowshi in astronomy and astrophys including cosmology a gravitational physics. T duration of a fellowship flexible within the range of to 5 years with the possibili of conversion to a tenur position. As a newly set institution IUCAA offe challenging opportunities experts in theory, observ tions as well as astronomic instrumentation.

Apply with curriculum vit and list of publications **Professor J.V. Narlikar, Di ector, IUCAA, Poona Universi Campus, Pune 411 007, Indi** Candidates should arrange send three confidential refe ences to this address. Review applications will begin on May 1989 for fellowships starting o September 1, 1989. (W5782)E



COMMISSION OF THE EUROPEAN COMMUNITIES

Directorate-General for Science, Research and Development

Postgraduate fellowships for Cancer Research Training

EUROPE AGAINST CANCER
In 1985 the Heads of State and the Governments of the twelve Member States of the European Community stressed the importance of launching a European programme in the fight against cancer.

In order to implement that conclusion of the European Council, a «Europe against Cancer» programme has been prepared.

Among the areas covered by this proposal for a plan of action is Cancer Research (cancer research training, clinical treatment research, epidemiological research, early detection and diagnosis, drug development and fundamental research).

The cancer research area will be developed within the framework of the fourth Medical and Health Research Programme, 1987-1991.

PARTICIPATING STATES
Due to the great interest in the cancer problem and in the «Europe against Cancer» programme, it is expected that European non-member States may decide to participate in the fellowship scheme. The participation of Switzerland and Austria is already certain.

FELLOWSHIPS
Approximately 50 postgraduate fellowships are available each year to scientists from any participating State who wish to work for some time in a centre for fundamental, clinical or epidemiological cancer research in another participating State. The fellowships may be either long-term (one year) or short-term (one to four months). The Selection Committee may award a two-year fellowship in cases of exceptional scientific merit.

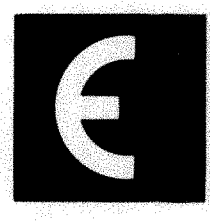
CONDITIONS OF ELIGIBILITY
- ☐ The award of fellowships is reserved to nationals of participating States.
- ☐ The research work must be carried out in a country other than the country of origin or the country of permanent residence.
- ☐ Submission of an application form and its annexes. The annexes must include a plan of research work approved by the intended host laboratory.

IMPLEMENTATION
The deadlines for applications in 1989 will be **Monday, 10 April** and **Monday, 9 October**; decisions will be taken by a panel of European Scientists in mid-June and mid-December respectively.

In general, applicants may begin work four months after a positive decision on their application.

INFORMATION
A «Guide to scientific and technical sectoral grants», including an application form, is available in all Community languages and may be obtained upon request from the address given below:

Commission of the European Communities,
Directorate-General for Science, Research and Development, (DG XII),
Division XII-H-1 «Grants» Action,
rue de la Loi 200, B-1049 BRUXELLES.
Tél: 32/2/235.39.91 or 235.40.67.



(W5789)E

BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY

FELLOWSHIP — 1989

The British Journal of Experimental Pathology offers a Fellowship in the field of experimental pathology to be awarded in the spring of 1989 and to be held for up to three years. This Fellowship is open to both medically and non-medically qualified applicants; preference will be given to applicants under the age of 40 years.

The stipend will be on the lower part of the lecturer scale and some allowance for consumables etc may also be made. The scheme is open only to applicants from institutions within the UK.

The closing date for applications is three weeks from the appearance of this advertisement. Applications should be addressed to **Professor N Woolf, Department of Histopathology, University College and Middlesex School of Medicine, The Bland-Sutton Institute, Riding House Street, London W1P 7AA.** (8177)E

NATIONAL KIDNEY RESEARCH FUND

RESEARCH FELLOWSHIPS

The National Kidney Research Fund (NKRF) is to offer two Fellowships, one clinical and one non-clinical in research areas related to renal disease. The Fellowship may be held in any suitable department in the UK. Fellows will be appointed for three years in the first instance, but an extension for a further two years will be possible. A grant of £5,000 per annum for research expenses will be available to the host department. The clinical Fellowships salary will be between £13,470 and £19,610 depending on experience. The non-clinical Fellowship salary will be at the appropriate post-doctoral level.

Closing date for receipt of applications 6th March 1989.

Application forms may be obtained from:

The Director-General, NKRF, 42 Lower Marsh, London SE1 7RG. (8099)E

CANCER RESEARCH POSTDOCTORAL FELLOWSHIPS IN MOLECULAR GENETICS, ENDOCRINOLOGY, IMMUNOLOGY, AND TUMOR BIOLOGY

The University of Rochester Cancer Center invites applications for post-doctoral positions in our research training program for 2 years beginning in 1989. The program is conducted in an integrated research and clinical setting and provides laboratory research training in a specific area of cancer research as well as a comprehensive exposure to the biology and biochemistry of cancer. Projects involve molecular cloning and structural analysis of genes related to cellular proliferation, the role of oncogenes in carcinogenesis and the molecular biology of carcinogen-activating drug metabolizing enzymes, molecular mechanisms of hormonal and nutritional regulation of the expression of specific proteins, characterization of tumor antigens and applications of hybridoma technology, the design and synthesis of new agents to increase cytotoxic drug selectivity, and the evaluation of heterogeneous cell populations in tumors and their influence on responses to therapy. Please send résumé and three letters of recommendation to: **Dr. Edgar Henshaw, University of Rochester Cancer Center, 601 Elmwood Avenue, Box 704, Rochester, NY 14642.** *The University of Rochester is an Equal Opportunity/Affirmative Action Employer.*

(NW3235)E

POSTDOCTORAL FELLOWSHIP

available now to study repair of 06-methylguanine in DNA and human tumor suppressor genes in the laboratory/clinical collaborative program of the Molecular Genetics and Carcinogenesis Laboratory of The Cross Cancer Institute. Two years with third possible. Background in DNA repair and molecular techniques advantageous. $23,815–$30,449.

Send curriculum vitae and names of three references to **Dr. Rufus S. Day, III, Cross Cancer Institute, 11560 University Ave., Edmonton, Alberta, T6G 122, Canada.** (NW3229)E

POSTDOCTORAL FELLOWSHIP OR JUNIOR FACULTY POSITION

The Lipid Research Division of Internal Medicine seeks a qualified M.D. and/or Ph.D. candidate to work in the molecular genetics area. Familiarity with classical as well as molecular genetics is required. A funded project with first year salary range of $24K-$26K depending on experience and expertise. Start date is 4/1/89 or soon thereafter. Project involves molecular genetics to ascertain defects of patients in apolipoproteins identified through an on-site screening program. Send CV/outline of research interests to:

Dr. Gustav Schonfeld, Washington University School of Medicine, Division of Lipid Research, 4566 Scott Ave., Box 8046, St. Louis, MO 63110.

(NW3195)E

Center for Neuroscience
University of Pittsburgh

Clinical Neuroscience Research Postdoctoral Fellowshi

Two- to three-year Fellowships for M
Ph.D.s interested in acquiring basic l
ratory skills to investigate neural disea
Fellowship should begin upon compl
of residency. Fellows will work with
basic science and clinical ment
$35,000 per year plus benefits. Write C
CNR Program, 337 E Scaife Hall, Uni
sity of Pittsburgh, Pittsburgh, PA 1526
detailed information and application

(NW3209

nature

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in science

STUDENTSHIPS

MRC Medical Research Council

MEDICAL RESEARCH COUNCIL
CLINICAL ONCOLOGY AND RADIOTHERAPEUTICS UNIT

Research Studentship in the Mechanism of Action of Novel Anticancer Drugs

A Studentship funded by the MRC is available from October 1989 Applications are invited from those who hold, or expect tc receive this summer, a first of upper second class degree in a relevant subject. We are seeking a highly motivated student whc will take advantage of the unique facilities available in this Unit and its location within the MRC Centre.

Available projects include:

1 Mechanism of action of membrane-interactive anticancer drugs.

2 Enzymology of bioreductive anticancer drugs.

Informal enquiries can be made by letter or phone to Dr P Workman (0223) 245133.

Applications quoting reference CO/RS, including the names of two academic referees, should be addressed to **The Personnel Officer, MRC Centre, Hills Road, Cambridge CB2 2QH.**

The closing date for applications is Friday 17 February 1989.

Smoking is actively discouraged in the Unit.

The Medical Research Council is an Equal Opportunity Employer

(8173)F

KING'S COLLEGE LONDON
University of London
BIOMEDICAL SCIENCES DIVISION
Postgraduate Studentships

The Biomedical Sciences Division expects to have a number of PhD studentships available from Research Councils and Medical Research Charities in the general areas of Anatomy, Pharmacology, Physiology, Cell Biology and Human Biology.

Students who have, or expect to obtain, an Upper Second Class Honours degree or better in a relevant subject in the June 1989 examinations and who are interested in these studentships should contact **Professor S L Howell, Biomedical Sciences Division, King's College, London, Campden Hill Road, London W8 7AH, for a list of potential topics and supervisors.** (8184)F

UNIVERSITY OF MANCHESTER

DEPARTMENT OF CHEMISTRY

GLAXO RESEARCH STUDENTSHIP IN SYNTHETIC ORGANIC CHEMISTRY

pplications are invited for a search studentship to work ith Professor E.J. Thomas on e total synthesis of a group of mplex biologically active acrocyclic compounds. Applitions should have, or expect to ave, at least a 2.1 honours egree in chemistry. The post is nded by Glaxo Group Reearch and the stipend will be at e postgraduate studentship vel, i.e. £3125 at present. pplications including a c.v. and e names and addresses to two ferees should be sent as soon possible to

rofessor E.J. Thomas, The epartment of Chemistry, The niversity, Manchester M13 PL, from whom further particurs may be obtained. (8218)F

CANCER RESEARCH CAMPAIGN EXPERIMENTAL CHEMOTHERAPY RESEARCH GROUP

PHARMACEUTICAL SCIENCES INSTITUTE ASTON UNIVERSITY BIRMINGHAM B4 7ET

POSTGRADUATE STUDENTSHIPS

FOUR or more PhD studentships are available in this Group, commencing in October 1989. The Group provides excellent training in methods of research, with five academics, eight postdoctoral fellows and funds in excess of £0.5 million per annum. Synthetic chemistry, molecular graphics, cell biology, molecular pharmacology, and preclinical toxicology are the main areas of interest of an integrated programme. The following project areas are open for 1989:

1. Studies of the biochemical mechanism of tumor cytokine-induced host catabolism involving characterisation of membrane signalling cascades responsible for changes in muscle protein degradation. Characterisation of the factors responsible for muscle protein degradation. Contact Dr Mike Tisdale.

2. Studies of colon carcinoma cell differentation: the role of tumor-extracellular matrix components in drug-induced differentation. The molecular biology of heat shock protein synthesis during leukemic cell differentation. Contact Dr John Hickman.

3. Studies of the modulation of protein kinase C isoforms by growth inhibitory agents. Contact Dr Andy Gescher.

4. Rational design of DNA sequence specific antitumor drugs directed to cytosine-guanine rich sequences in oncogenes. Contact Professor Malcolm Stevens.

Candidates must hold or be expecting to obtain a first or upper second class honours degree. A number of these studentships are offered at a premium level of reimbursement. For further details contact the appropriate person in each of the areas shown ENCLOSING a full curriculum vitae and the names of two academic referees by March 1st 1989.

ASTON UNIVERSITY

(8254)F

LUDWIG INSTITUTE FOR CANCER RESEARCH

MIDDLESEX HOSPITAL/UNIVERSITY COLLEGE BRANCH

PhD Studentship

The Institute is a superbly equipped Research Laboratory (Director Dr M D Waterfield) located in a newly refurbished building close to the Middlesex Hospital. There are seven groups working in the general areas of growth control and cell signalling in relation to carcinogenesis, differentiation and development. PhD Studentships are available beginning in October 1989. The group leaders and their research interests are as follows:—

PETER PARKER: the structure and function of signal transducing molecules and their role in 'normal' and neoplastic growth.

JEREMY BROCKES: molecular basis of cell patterning in limb development and regeneration.

MICHAEL WATERFIELD: structure and function of growth factors and receptors.

PAUL STROOBANT: purification and analysis of growth and differentiation factors, and their role in initiation, growth and metastasis of tumour cells.

MARK NOBLE cellular and molecular control of development, regeneration and neoplasia in the central nervous system; oncogene function and regulation of differentiation.

JIM WOODGETT: analysis of growth regulatory molecules: emphasis on regulators of oncogenes and transducers of growth factors.

PARMJIT JAT: molecular regulation of cell growth, immortalisation, transformation and senescence.

Interested students should apply as soon as possible to:

SECRETARY TO THE ADMINISTRATOR
LUDWIG INSTITUTE FOR CANCER RESEARCH
91 RIDING HOUSE STREET
LONDON W1P 8BT
Tel: 01-436 8441

(8223)F

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

SERC CASE STUDENTSHIP IN MOLECULAR TOXICOLOGY

DEPARTMENT OF SURGERY

Applications are invited for candidates holding or expecting to obtain this summer an honours degree (at least 2/1 standard) in an appropriate discipline for a SERC funded CASE studentship in collaboration with the Central Toxicology Laboratory, ICI, Macclesfield, Cheshire (industrial collaborator, Dr John Foster).

The research project will be to study the regulation of expression of cell surface receptors in both normal and toxin-damaged hepatocytes. The project will primarily require a sound knowledge of contemporary molecular biology techniques and some practical experience in this field would be an advantage.

Applications, comprising a curriculum vitae and the names of two academic referees, should be sent to:—

Dr David Grant, Dept. Surgery, St George's Hospital Medical School Cranmer Terrace, London SW17 0RE, and from whom further details maybe obtained (01-672 9944) ext. 55590). Reference advert: 11-89. (8199)F

NATIONAL KIDNEY RESEARCH FUND

STUDENTSHIPS

The National Kidney Research Fund (NKRF) is to offer three Studentships in research areas related to renal disease. Heads of department are invited to apply for studentship allocations. Student stipends vary between £3,690 p.a. (outer London) £4,500 (London) plus approved student fees and contribution to departmental research expenses.

Closing date for receipt of application 6th March 1989.

Application forms may be obtained from:

The Director-General, NKRF, 42 Lower Marsh, London SE1 7RG.

(8100)F

PATERSON INSTITUTE FOR CANCER RESEARCH
Christie Hospital & Holt Radium Institute

Cancer Research Campaign Studentships

Five postgraduate studentships will be available in the Paterson Institute starting 1st October 1989. These studentships are for 3 years and carry a grant from the CRC of £4,458 per annum (subject to review). The projects are:—

1. Growth factor expression by intestinal epithelial cells and the role of these factors in maintaining the stem cell niche within the intestinal crypts using both established intestinal cell lines and fractions of fresh intestinal cells enriched for crypt and villus cells. Supervisors Dr G.S. Evans (Epithelial Biology) & Dr M. Mackett (Molecular Biology).
2. Cloning and expression of eukaryotic and viral genes involved in DNA repair, using recombinant DNA technology, gene transfection, and assessment of the effect of the recombinant genes on damage caused by carcinogenic and mutagenic agents. Supervisors Dr E. Littler (Molecular Biology) & Dr G.P. Margison (Carcinogenesis).
3. Investigation of the mechanism of action of some novel anti-cancer drugs which are believed to disrupt cellular tubular function, thus providing an opportunity to study these functions in both normal and tumour cells. Applicants should have a strong interest in development of new anti-cancer drugs. Supervisors Dr A. McGown & Professor B.W. Fox (Experimental Chemotherapy).
4. Isolation and characterisation of genes encoding cell surface markers of cervical neoplasia using molecular biological techniques and functional studies on the modulation of the immune response. Supervisor Dr P.L. Stern (Immunology in collaboration with the Molecular Biology group).
5. Identification and characterisation of the growth factors, cell adhesion molecules and extracellular matrix components which are involved in cellular interactions during haemopoiesis, with particular emphasis on the regulation of stem cell growth and development. Techniques will include molecular cloning and gene expression, biochemistry and cell culture. Supervisors Professor T.M. Dexter (Haematology) Dr J. Gallagher (Medical Oncology).

The Paterson Institute for Cancer Research is a multidisciplinary research centre with all the major facilities and many research opportunities.

Applicants should have or expect to gain a first or upper second class honors degree in an appropriate subject. They should indicate which studentship(s) are of interest and submit a detailed CV including the names of two academic referees to Professor D.G. Harnden, Director, Paterson Institute for Cancer Research, Christie Hospital & Holt Radium Institute, Wilmslow Road, Manchester M20 9BX.

Closing date 24th February 1989. (8222)F

ASSISTANTSHIPS

CAMBRIDGE UNIVERSITY
Institute of Biotechnology

YEAST MOLECULAR BIOLOGY

A three year post-doctoral research assistantship is available to work on the molecular basis of gene regulation and copy number control in the yeast 2μ plasmid.

Experience in molecular biology or yeast genetics would be an advantage. Salary scale £10,460–£11,680. Earliest starting date is April 1989. Please send applications, consisting of a cv with the names of two referees and a description of research experience, as soon as possible, but in any case by 28th February 1989 to:

Dr. Jim Murray
University of Cambridge
Institute of Biotechnology
Downing Street, Cambridge CB2 3EF

Informal telephone enquiries are welcome on 0223 334754. Fax 0223 334734 (8242)P

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF CLINICAL VETERINARY MEDICINE
ANIMAL WELFARE GROUP

RSPCA RESEARCH ASSISTANTSHIP

Applications are invited for the post of Research Assistant to compile a review of stress measurements in laboratory animals. The work would be for two years. It would involve a literature survey, the evaluation of procedures and the production of guidelines. Applicants should have a Ph.D., other research experience, or veterinary or medical qualifications and should be under 26 years of age or prepared to work part-time. The salary will not be more than £10,460 per annum.

Apply enclosing curriculum vitae and the names of two referees before 28th February, 1989 to **Professor D.M. Broom, Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES.** (8206)P

KING'S COLLEGE LONDON
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANTSHIP IN NUCLEOTIDE CHEMISTRY

Applications are invited from organic chemists for a Postdoctoral Research Assistantship. The successful candidate will be required to work under the supervision of Professor C B Reese on the synthesis of oligo- and poly-nucleotides.

The Assistantship will be tenable for one year in the first instance. Salary on the 1A scale £9,865 – £11,680 per annum + £1,650 per annum London Allowance.

Applications including a **Curriculum Vitae** and the names and addresses of two referees, should be sent to: **Professor C B Reese, FRS, Department of Chemistry, King's College London, Strand, London, WC2R 2LS.**

Closing date for receipt of applications is 17th February, 1989. (8232)P

AGRICULTURAL & FOOD RESEARCH COUNCIL
Institute of Animal Physiology and Genetics Research
Cambridge

RESEARCH STUDENTSHIP
CELLULAR SIGNALLING

An MRC-funded Research Studentship, leading to a PhD in the University of Cambridge, is available starting in October 1989 to study the application of Molecular Biology to Cellular Signalling. The project, to be supervised by Dr R F Irvine in collaboration with Dr K A Wreggett in the Department of Biochemistry, is designed to study the physiology of the phosphoinositide signalling system in intact animals and cells, and will involve techniques in molecular biology. Prior technical knowledge of molecular biology or phosphoinositides is not required.

The Institute provides an excellent academic environment for research training with facilities at the leading edge of technology and currently has over 30 PhD students. Applicants should hold or expect a 1st or upper second class degree in a relevant subject. Applicants, including a curriculum vitae naming two referees, should be submitted as soon as possible to Dr R J Bicknell, Tutor for Research Students, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT. Informal enquiries to Drs Irvine or Wreggett are welcomed (Tel: 0223 832312). (8247)P

MEDICAL RESEARCH COUNCIL
MRC MAMMALIAN DEVELOPMENT UNIT

STUDENTSHIP

Applications are invited for three year MRC studentship starting in October 1989, an leading to a PhD degree the University of Londo The research under the supe vision of Dr Paul Burgoyn would focus on mammalian chromosome function. Appl cants should have, or expect t obtain, a good honours degre in a biological subject, prefe ably including some molecula biology and genetics.

Curriculum vitae with you daytime telephone numbe and names and addresses c two referees, should be sent t The Secretary, MRC Mamma lian Development Unit, Wol son House, 4 Stephenso Way, London NW1 2HE (tele phone 01 387 9521).

(8245)P

MRC
Medical Research Council

GRANTS & SCHOLARSHIPS

THE NUFFIELD FOUNDATION OLIVER BIRD FUND FOR RESEARCH INTO RHEUMATISM

Applications are invited for research grants for work directed to the better understanding, and treatment, of rheumatic disease. The Oliver Bird Fund, from which over £200,000 was spent on research grants during 1988, is administered by the Trustees of the Nuffield Foundation on the advice of an expert committee whose chairman is Dr D A J Tyrrell, CBE, FRS. The Committee is particularly interested in receiving applications from younger researchers, especially those at the early stages of their independent research careers.

Further details of how to apply are available from the Nuffield Foundation.

The closing date for applications is 31st March 1989.

Applications will be considered at a meeting in May 1989.

Grants may be made only for research in UK institutions.

Nuffield Foundation, 28 Bedford Square,
London WC1B 3EG. (8186)H

AWARDS

The Protein Society
Award Nominations Solicited

1989
THE DUPONT AWARD

This new award is for an important contribution to the study of proteins by a scientist not yet 38 years of age at the time of his or her nomination. The awardee will be honored at the 1989 Symposium of the Society by presenting a plenary lecture and receiving a prize of $6000 and a scroll. The DuPont Award is supported through the generosity of E.I. DuPont & DeNemours Co.

1990
THE STEIN & MOORE AWARD

This award of the Society recognizes contributions at the highest level to the study of proteins. The awardee will be honored at the 1990 Symposium of the Society by a session in his or her honor, and will receive a prize of $7500 and a scroll.

Deadline for nominations for either award: April 1, 1989

Please send documented nominations to:
Professor Christopher T. Walsh, Chair, Awards Committee.
Department of Biological Chemistry and Molecular Pharmacology
Harvard Medical School, 240 Longwood Av., Boston, MA 02115, USA.

SYMPOSIUM: The 1989 Symposium will be held in Seattle, WA, July 29 through August 2. The tentative program and application forms are available from **Teresa Spellman, Conference Management GH-22, University of Washington, Seattle, WA 98195, (206) 543-2300.**

WORKSHOP: A special 3D Structure Prediction Workshop is planned, at which predictions will be compared to actual structures. The Protein Society invites participation of scientists with new, as yet unpublished structures, and scientists involved in methods of structure prediction. Please contact **Dr. Douglas Rees, Molecular Biology Institute, UCLA, Los Angeles, CA 90024, USA.**
BITNET address: REES%UCLAUE.SPAN@STAR.STANFORD.EDU.
(NW3242)N

SEMINARS & SYMPOSIA

International Meeting

NITRIC OXIDE FROM L-ARGININE: A BIOREGULATORY SYSTEM

14th/15th September 1989
at The Royal Society, London, England

Evidence now emerging from various unrelated fields of research is revealing the existence of a biochemical pathway for the formation of nitric oxide from the amino acid L-arginine. This meeting will be an up-to-date discussion of the biological implications of nitric oxide formation.

Participants include:

Prof. A. Barbul (USA)
Prof. R. Furchgott (USA)
Dr. J. Garthwaite (UK)
Dr. R.M.J. Palmer (UK)
Prof. J.B. Hibbs, Jr. (USA)
Prof. M.A. Marletta (USA)
Prof. F. Murad (USA)

Attendance will be limited to 150. Prospective participants are asked to apply to the organiser for further details and abstract forms by 13th March, 1989.

Organiser:

Dr. S. Moncada, F.R.S.,
The Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent BR3 3BS, U.K.
Telephone: 01-658 2211 Fax: 01-658 2278
(8172)M

The Third Symposium of
The Protein Society
will be held
July 29–August 2, 1989 in Seattle, WA

Plenary Speakers:
J. Richardson, B. Matthews, R. Huber, H. Schachman, E. Davie
Stein/Moore Symposium: H. Neurath, P. Walter, R. Jaenicke, E. Fischer

TENTATIVE PROGRAM

Protein Folding (D. Shortle, K. Dill, P. Kim)
Glycoproteins (A. Dell, A. Varki, J. Hart)
Semisynthetic Enzymes (P. Dervan, P. Schultz)
Transducing Proteins (M. Simon, C. Shutt, H. Berg)
Protein/Nucleic Acid Interaction (P. Sigler, J. Berg)
Ubiquitin, Protein Degradation (A. Hershko, J.F. Dice, A. Varshavsky)
Viral Proteins (S. Harrison, R. Hendrix, M. Rossmann)
Intracellular Structures (K. Wang, S. Brady, J. Scholey)
Chimeric Proteins (R. Lefkowitz, K. Yamamoto)
Enzyme Inhibitors (P. Bartlett, J. Kraut, D. Christianson)
RNA Recognition & Processing (J. Abelson, P. Schimmel)
Membrane Transport (G. Blobel, J. Beckwith, H. Lodish)

WORKSHOP

Predictions of 3D structures will be compared to actual structures. The Protein Society invites participation of scientists with new, as yet unpublished structures, and scientists involved in methods of structure prediction.

Please contact **Dr. Douglas Rees, Molecular Biology Institute, UCLA, Los Angeles, CA 90024, USA. BITNET address: REES%UCLAUE.SPAN@STAR.STANFORD.EDU.**

FOR SYMPOSIUM INFORMATION AND APPLICATION FORMS
Please contact Teresa Spellman, Conference Management GH-22
University of Washingon, Seattle, WA 98195 (206)543-2300
(NW3243)M

The University of Texas M. D. Anderson Cancer Center and Smith Kline & French Laboratories

Announce

AN INTERNATIONAL SYMPOSIUM

CRITICAL DETERMINANTS IN CANCER PROGRESSION AND METASTASIS

March 6-10, 1989

J. W. Marriott Hotel at the Galleria • Houston, Texas

PROGRAM:

M. Anzano	P. Donahoe	D. Gospadarowicz	I. Krakoff	J. Massague	R. Ross
C. Balch	A. Dunn	R. Greig	M. Kripke	T. McKearn	V. Schirrmacher
F. Becker	M. Feldman	I. Hart	R. Ladda	H. Moses	M. Sporn
M. Bissel	I. Fidler	G. Heppner	V. Ling	G. Nicolson	E. Stanbridge
T. Boon	J. Folkman	R. Hynes	L. Liotta	P. Nowell	I. Tannock
W. Clark	P. Frost	R. Jain	M. Lippman	G. Poste	D. Tarin
G. Cunha	L. Furcht	C. Jordan	D. Louvard	M. Raber	B. Vogelstein
J. Dennis	R. Goodenow	R. Kerbel	G. Martin	A. Raz	S. Yuspa

For Additional Information and Registration Materials, Please Contact
Pam Evans • Office of Conference Services–HMB 131
UT M. D. Anderson Cancer Center
1515 Holcombe Boulevard • Houston, Texas 77030
(713)792-2222

(NW3185)M

5th Annual Symposium
Molecular Biology of Hematopoiesis

University of Innsbruck, July 10-12, 1989

TOPICS:

Hematopoietic Growth Factors, Gene Transfer, Growth Factors and Stroma, Abnormal Hematopoiesis, Experimental Models of Leukemia, Gene Regulation in Hematopoiesis, Differentiation, Therapeutic Use of Hematopoietic Growth Factors.

SPEAKERS:

Abraham N, USA; Adamson J, USA; Ascensao J, USA; Bagby BC, USA; Bartram CR, FRG; Benz E, USA; Beug H, Austria; Broxmeyer H, USA; Bunn H, USA; Clark S, USA; Crowther D, UK; Eaves C, Canada; Forget B, USA; Golde D, USA; Grandchamp B, France; Grosveld F, UK; Grosveld G, Netherlands; Hoffman R, USA; Jacobs A, UK; Keller G, Austria; Koeffier P, USA; Konwalinka G, Austria; Marks P, USA; Marshall C, UK; Metcalf D, Australia; Miller A, USA; Moore M, UK; Mulligan H, USA; Naguchi T, Japan; Nicola N, Australia; Nienhuis A, USA; Orkin S, USA; Ostertag W, FRG; Peschie C, Italy; Plumb M, UK; Pragnell I, UK; Quesenberry P, USA; Raz A, USA; Sachs L, Israel; Schwartzmann M, USA; Segal G, USA; Sieff C, USA; Spooncer E, UK; Tambourin P, France; Wagner W, Austria; Wiedemann L, UK; Wright E, UK; Zanjani E, USA.

Registration Fee: US Dollars 150.00 until April 28th, 1989.
Abstract deadline: April 28th, 1989.

For program details and registration form apply to:

G. Konwalinka, Dept. Internal Med., Univ. Innsbruck, 6020 Innsbruck, Austria (Tel: 5222-504 3255)

or

N. Abraham, New York Med. Coll., Valhalla, 10595 NY, USA (Tel. 914-993 4426).

(W5804)M

5th FAOB CONGRESS
Seoul, Korea
August 13 – 18, 1989

The scientific program is composed of seven plenary lectures, seventeen symposia in various fields of biochemistry, colloquia, and poster sessions.

Invited speakers will include distinguished scholars from all parts of the world.

Colloquia and poster sessions are open to all participants for presentation of their research results.

BIOEXPO'89 SEOUL will be organised during the Congress. Submission of abstract is due by March 1.

For information, please contact: 5th FAOB Congress, Secretariat, c/o Korea Convention Services Ltd., K.P.O. Box 233 Seoul 110-602 Korea. Phone: 82-2-739-2141/4. Fax: 82-2-739-2145.

(W5794)M

MISCELLANEOUS

NUMBERS are adjectives, but nouns are sets in space and verbs are sets in time, meaning being their intersection. Separated in the retina they are reunited in speech. Linguistics is a branch of mathematics. Box 8197 c/o Nature 4 Little Essex Street London WC2R 3LF. (8197)J

APPTS. WTD

ELECTRON MICROSCOPIS Ph.D. (Entomology), Po Doc. (Heavy Metals), 2 Extensive experience E.M related techniques see position in E.M.; Industr Medical/Academia, Australi N.Z. pref. Box No. 8230B c Nature, 4 Little Essex St London WC2R 3LF.

(8230)B

AACR SPECIAL CONFERENCES IN CANCER RESEARCH

Gene Expression in Cancer Cells

May 22-24, 1989, San Francisco Hilton, San Francisco, CA
Chairman: Inder M. Verma, Salk Institute, San Diego, CA

Topics: suppressor oncogenes, *muc*, EGF receptor, E1A, and cyclic-AMP regulation, *fos, jun, mos.*

Invited Speakers: Webster K. Cavenee, Mark Groudine, Joseph Schlessinger, Thomas E. Shenk, Inder M. Verma, George F. Vande Woude, Jr.

Molecular Events in Mutation and Cancer

May 21-23, 1989, Tiburon Lodge, Tiburon (Marin County), CA
Co-Chairmen:
B. Singer, University of California, Berkeley, CA
D. J. Patel, Columbia University College of Physicians and Surgeons, New York, NY

Topics: single base mismatches, O-Alkyl G and T, cyclic and bulky adducts, abasic sites and extrahelical bulges.

Invited speakers and discussion leaders: H. M. Berman, D. M. Crothers, J. M. Essigmann, M. F. Goodman, A. P. Grollman, D. Grunberger, L. H. Hurley, K. W. Kohn, S. J. Lippard, D. J. Patel, A. E. Pegg, L. Ripley, B. Singer, B. S. Strauss, J. A. Swenberg, I. Tinoco, Jr., I. B. Weinstein, R. D. Wells

Molecular Aspects of Growth Control

(Joint AACR/Japanese Cancer Association Meeting)
May 28-31, 1989, Sheraton Waikiki Hotel, Honolulu, HI
Co-Chairmen:
Enrico Mihich, Roswell Park Memorial Institute, Buffalo, NY
Takashi Sugimura, National Cancer Center, Tokyo, Japan

Topics: oncogene and cancer cell phenotypes, cytokines and regulatory factors, gene function and the carcinogenic process, control mechanisms and cancer therapeutics.

Invited speakers and discussion leaders: Ruth Sager, Hidesaburo Hanafusa, Haruo Sugano, Inder M. Verma, Charles J. Sherr, Kumao Toyoshima, Mitsuaki Yoshida, Masaaki Terada, Michel Nussenzweig, Yuichi Yamamura, Tony Hunter, William J. Rutter, Joan Massague, E. Richard Stanley, Tadatsugu Taniguchi, Tadamitsu Kishimoto, Wataru Mori, I. Bernard Weinstein, Mariano Barbacid, Frederick W. Alt, Robert A. Weinberg, Susumu Nishimura, Hirota Fujiki, Takashi Sugimura, Hiroshi Kabayashi, Ira Pastan, Yoji Ikawa, Malcolm A. S. Moore, Alexander Bloch, Takashi Tsuruo, Fuminaro Takaku, Masami Muramatsu, Enrico Mihich

The Cell Membrane and Cell Signals as Targets for Cancer Chemotherapy

(Joint AACR/BACR/EORTC [PAM] Meeting)
September 14-16, 1989. Queens' College, Cambridge, UK
Chairman: Garth Powis, Mayo Clinic and Foundation, Rochester, MN

Topics: membranes and signalling targets, physical interaction of membranes, growth factor initiated signalling, membrane lipids and signalling, ions, protein kinases and cellular differentiation, metastasis and cell death.

Invited Speakers: Jean-Pierre Abita, Wofgang Berdel, Michael Berridge Michael D. Cahalan, Dennis Chapman, David Clapham, Benjamin de Kruijff, T. Michael Dexter, Peter Downes, Janet G. Dzubow, Gordon Foulkes, Andreas Gescher, Yosef Graziani, Hans H. Grunicke, Ian R. Hart, John A. Hickman, Marc Mareel, Christopher Marshall, Edward J. Modest, Wouter Moolenaar, Harold L. Moses, Garth L. Nicolson, Ewa Ninio, Alexander Noseda, Sten Orrenius, Peter J. Parker, Garth Powis, Timothy J. Rink, Enrique Rozengurt, Thomas R. Tritton, Michael D. Waterfield.

Information and Application Forms

American Association for Cancer Research
530 Walnut Street, 10th Floor
Philadelphia, PA 19106
215-440-9300
FAX: 215-440-9313

(NW3238)C

NATO Advanced Study Institute

Sulphur — centred Reactive Intermediates in Chemistry and Biology

MARATEA (Italy)
June 18-30, 1989

Director:

C. CHATGILIALOGLU
I.Co.C.E.A – C.N.R.
Via della Chimica, 8
I - 40064 OZZANO EMILIA
Bologna
Italy

Associate Director:

K.-D. ASMUS
Hahn – Meitner – Institut
Postfach 39 01 28
D - 1000 BERLIN 39
Federal Republic of
Germany

The main objectives of the school are to present the state-of-the-art in advanced interdisciplinary research on sulphur-centered reactive intermediates and to foster international scientific contacts by means of high-level teaching courses not yet in university curricula

****Further information can be obtained from the directors****

(W5803)C

Second Announcement
20th Anniversary of Insulin
Crystal Structure Determination

CONFERENCE

on

INSULIN

Structure, Chemistry & Biology

29th August – 1st September 1989
UNIVERSITY OF YORK
ENGLAND

PROGRAMME

Insulin Structure, Chemistry and Function
Insulin Evolution
Receptors of Insulin and Related Molecules
Insulin Biosynthesis and Secretion
Insulin Immunology
Insulin and Diabetes

For further information, registration and abstract forms contact:
Professor Guy Dodson, Department of Chemistry,
University of York, Heslington, York, YO1 5DD, UK

ABSTRACT DEADLINE: 7 APRIL, 1989

The conference is being supported by
NOVO Research Laboratories, Denmark. LILLY, USA.
NORDISK, Denmark. (8220)C

PRACTICAL COURSE IN

IMMUNOCYTOCHEMISTRY

DATE: 18-21st JULY 1989

This four day residential course is aimed at providing knowledge and hands on experience in the preparation, sectioning, and staining of tissues using immunofluorescence, immunoenzyme, immunogold and relevant enhancement techniques. The session will include lectures from established workers in the field as well as full instruction in the interpretation of results and production of photomicrographs.

The course fee will be £330. inclusive of excellent accommodation, meals, and V.A.T. The course places are limited and early application is warranted.

For further details please contact:

I. L. BETHELL
CONSULTANCY AND DEVELOPMENT UNIT
SOUTH GLAMORGAN INSTITUTE OF HIGHER EDUCATION
LLANDAFF CENTRE WESTERN AVENUE
CARDIFF CF5 2YB
WALES U.K.

Telephone (0222) 560685; (0222) 551111 (Ext. No. 4302) Fax (0222) 747208 (Attn. Consultancy Unit). (8214)C

CONFERENCE

The Second International Workshop on Cytokines
10 to 14 December 1989
Hilton Head, South Carolina

GOALS: a) to encompass and integrate the most recent data concerning cytokine synthesis, release, regulation, actions, and interactions, and b) to assess the in vivo effects of these cytokines on cell, tissue, organ, and organism metabolism, physiology, and immunity. TOPICS: structure, gene expression, regulation of synthesis and release, receptors and post receptor events, cytokine activities, alone and in combination, assays for and inhibitors of the cytokines. CYTOKINES TO BE CONSIDERED: the interleukins, the interferons, TNF, TGF, PDGF, chemotactic factors, the various colony stimulating factors, bone and connective tissue growth factors, as well as newly identified activities/factors.

INVITED SPEAKERS: Robert Friedman (interferons), Tadamitsu Kishimoto (IL-6), Thomas Maciag (ECGF), Lloyd Old (TNF), Russell Ross (PDGF), Ruth Sager (GRO), Arthur Silverstein (historical perspective). The content of the rest of the meeting will be based on submitted abstracts.

Abstracts will be published in the premiere issue of CYTOKINE (W B Saunders Company). Deadline for receipt of abstracts is 15 June 1989. For abstract forms, meeting information, and registration forms contact: **Sherwood M. Reichard, Cytokines, c/o RES Society, Box 3044 MCG, Augusta, GA 30912 USA.** (NW3168)C

University of Bristol
Department of Obstetrics and Gynaecology

MSc Degree Course in 'Human Fertility and Infertility'

A one year course starting in October 1989 to train doctors and laboratory scientists to conduct modern clinical and/or laboratory practice in Reproductive Medicine. It will provide advanced tuition in reproductive biology and medicine including endocrinology, ovulation induction, seminology, artificial insemination, tubal disease and *in vitro* fertilisation, and offer practical instruction in relevant clinical and laboratory techniques.

Further details may be obtained from: **MSc Course Secretary, University Department of Obstetrics and Gynaecology, Bristol Maternity Hospital, BRISTOL BS2 8EG, UK. Telephone (0272) 215411 Ext 5293.**

Application deadline: 1 June 1989. (8244)C

Two International Conferences Organised by IBC Technical Services Ltd

THE CLINICAL IMPACT OF INTERLEUKINS

10th/11th April 1989
Royal College of Physicians, London

With the first of the interleukins fast approaching the market, research on this group of proteins is now beginning to bear fruit. This international conference will examine the clinical data accumulated to date on interleukin-2 and on interleukin-2 receptor monoclonal antibodies, and review the latest data available on the other interleukins, from IL-1 to IL-7, with a look also at IL-8, IL-9 and beyond. An international panel of speakers will assess the clinical impact each of these agents is likely to have, and also the prospects they offer for new drug development.

Chairmen and Speakers to include:

Professor T. Hamblin
Royal Victoria Hospital, Bournemouth, UK

Professor S. Meuer
Deutsches Krebsforschungszentrum Heidelberg, W. Germany

Dr. R. Fildes
Cetus Corporation, USA

Dr. C. Mawas
INSERM, France

Dr. C. Henney
Immunex Corporation, USA

Dr. R. Callard
London Institute for Child Health, UK

Professor M. Feldman
Charing Cross Sunley Research Centre, UK

Dr. J. Schrader
Biomedical Research Centre, Vancouver, Canada

Dr. S. Gillis
Immunex Corporation, USA

Dr. J. Gauldie
McMaster University, Canada

Dr. L. Aarden
Netherlands Red Cross Blood Transfusion Service

Dr. C. Sanderson
National Institute for Medical Research, UK

NEW STRATEGIES FOR DEVELOPING PSYCHOTROPIC DRUGS

24th/25th April 1989
Royal College of Physicians, London

Most of the archetypes of the psychotropic drugs in use today were discovered by serendipity in the 1950s or 1960s. Since then, modern methods of drug screening have proven to be very effective in optimising these structures, but are these methods as efficient for finding new structural archetypes? At this symposium, a wide range of international experts who are tackling psychotropic drug research from a number of different angles will present their approaches, philosophies and experiences. Discussion will cover the use and limitations of structure/activity relationships, wide-ranging receptor binding as a screening strategy, the place of phytochemistry and neuroendocrinology, and the use of simple animal models and human behavioural psychopharmacology in the search for new psychotropic drugs.

Speakers will include:

Dr. M. Briley
Pierre Fabre Medicament, France

Professor G.R. Marshall
Washington University, St. Louis, USA

Professor W.D. Ollis
University of Sheffield, UK

Dr. C. Kordon
INSERM, France

Professor B. Costall
University of Bradford, UK

Professor C.G. Wermuth
University Louis Pasteur, Strasbourg, France

Dr. S. Garrattini
Istituto di Recerche Farmacologische Mario Negri, Italy

Dr. R. Porsolt
ITEM, France

Professor D. Grahame-Smith
University of Oxford, UK

Dr. S. File
Guy's Hospital, London

For a full details about both conferences, please contact:
Renata Duke, IBC Technical Services Ltd.,
Bath House (3rd Floor), 56 Holborn Viaduct,
London EC1A 2EX. Tel: 01-236 4080
Telex: 888870 IBC G Fax: 01-489 0849

(8246)C

PRACTICAL COURSES IN MOLECULAR BIOLOGY AT UNIVERSITY COLLEGE LONDON

Department of Biochemistry

(1) PRACTICAL INTRODUCTION TO GENETIC ENGINEERING
10 April to 14 April, 1989 (inclusive)
A one week full time practical course designed to teach the basic techniques in genetic engineering. DNA will be cloned in a multicopy plasmid vector and analysed intensively using restriction enzyme mapping, Southern hybridization, and DNA sequencing. The "hands-on" approach will enable scientists from higher education and industry, clinicians and research workers who have had no practical experience of recombinant DNA techniques to set up such techniques in their own laboratories. There will be lectures supplementing the practical work and covering other areas of molecular biology.
Course Fee £550 (exclusive of accommodation)
Numbers will be limited. Deadline for Applications: 6th March, 1989

(2) cDNA CLONING: A PRACTICAL COURSE
26 June to 30 June, 1989 (inclusive)
An intensive one-week laboratory course in the techniques of cDNA cloning. The practical course will include (a) the construction of a cDNA library in λgt 10, (b) the screening of λgt 11 libraries using both antibody and radiolabelled DNA probes, (c) detailed restriction mapping of cloned genes. In addition to the practical sessions there will be lectures discussing the methods which will allow efficient construction and screening of cDNA libraries and their application to biological, medical and biotechnological problems.
Course Fee £750 (exclusive of accommodation)
Numbers will be limited. Deadline for applications: 30th May, 1989.
Bed and breakfast accommodation can be provided for the period of either course in a local hall of residence for £84.
Write for application forms and further details to:
H.A. White, Department of Biochemistry
University College London
Gower Street, London WC1E 6BT

(8243)C

PROTEIN PURIFICATION COURSES

Practical Course Programme 1989
— European Consultancy —

1. **Protein Purification, Process Development, Optimization and Scaling up**
   4-day course leading to production scale chromatography (50 l columns)

2. **Automated Monoclonal Purification (Process Scale)**
   2-day course using new dedicated monoclonal purification media, software package, and instrumentation
   April 24–25; April 27–28; May 8–9; May 11–12, 1989

3. **Intensive Course on Strategies of Recombinant Protein Purification (Hands on)**
   4-day practical including process development, optimization, scale up, and analytical purity checking techniques
   May 29–June 2, 1989

For further information please contact:
Pharmacia
— European Consultancy —
Munzinger Straße 9,
D-7800 Freiburg
Telephone: 0049–761/4903–152
Telefax: 0049–761/4903–159
Telex: 7721 513 ph d

Pharmacia
European Consultancy
Process Chromatography Centre

(W5788)C

THE WELLCOME TRUST • Medical Research & History •

Summer School 1989

Two intensive, post-doctoral, residential, laboratory based courses will be available:

DNA related methods in Human Genetics

Co-directors: Kay E. Davies, Anna-Maria Frischauf, William Brown.

Lecturers: Duncan Campbell, Nick Hastie, Martin Hooper, Ed. Southern, Paul Travers.

July 14-23rd, 1989 (closing date for applications, March 10th).

Molecular Parasitology

Co-directors: Murray Selkirk, Debbie Smith, Julian Crampton.

Lecturers: Stephen Beverley, John Boothroyd, Alan Fairlamb, John Scaife, Ken Stuart, David Walliker.

September 1-10th, 1989 (closing date for applications, March 31st).

Applications in the form of a **curriculum vitae** and **one-page** summary of research should be sent to:

Dr. Pelin Faik, Wellcome Laboratory for Molecular Genetics, Division of Biochemistry, UMDS, Guy's Campus, London SE1 9RT.

(8256)C

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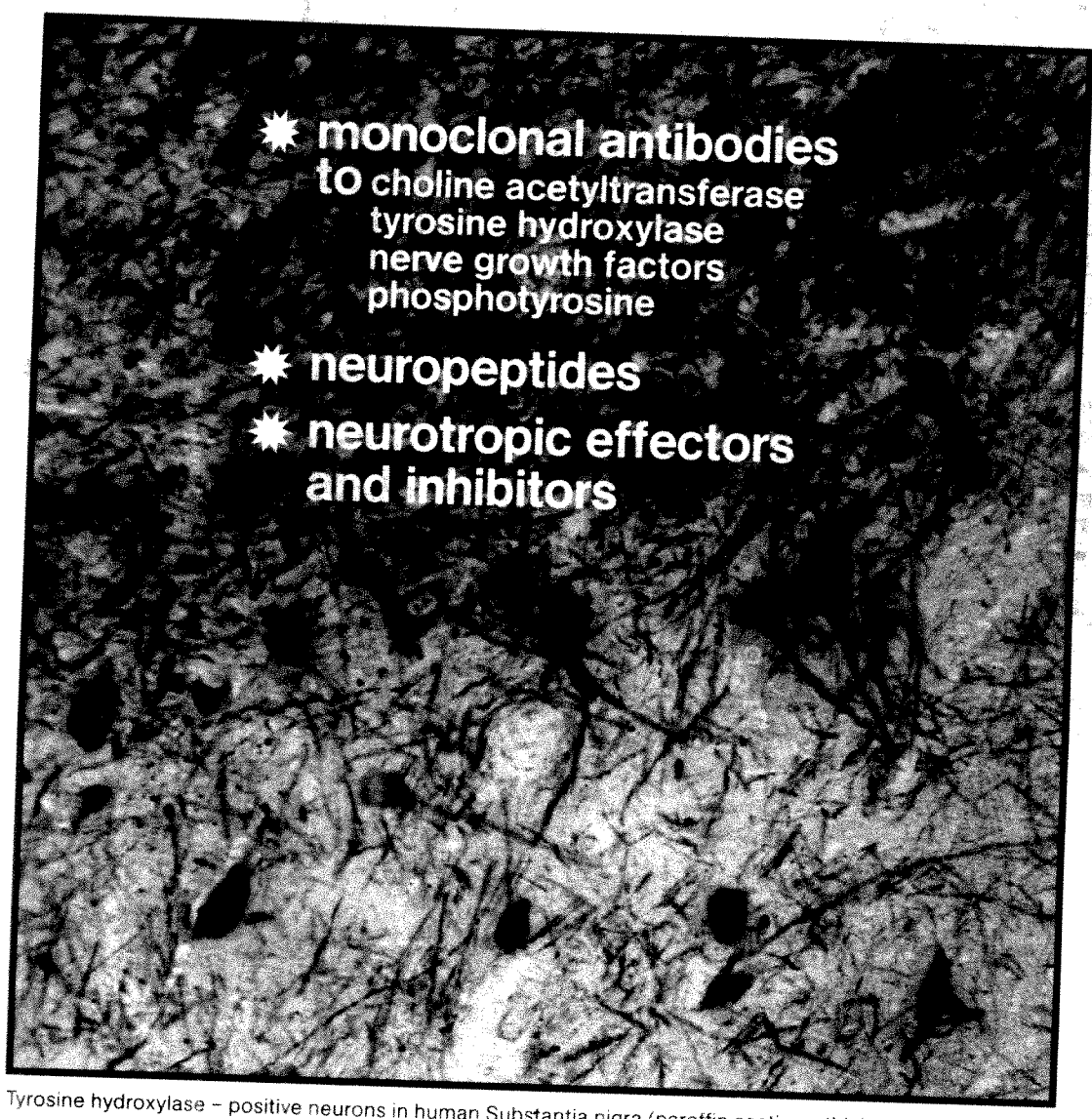
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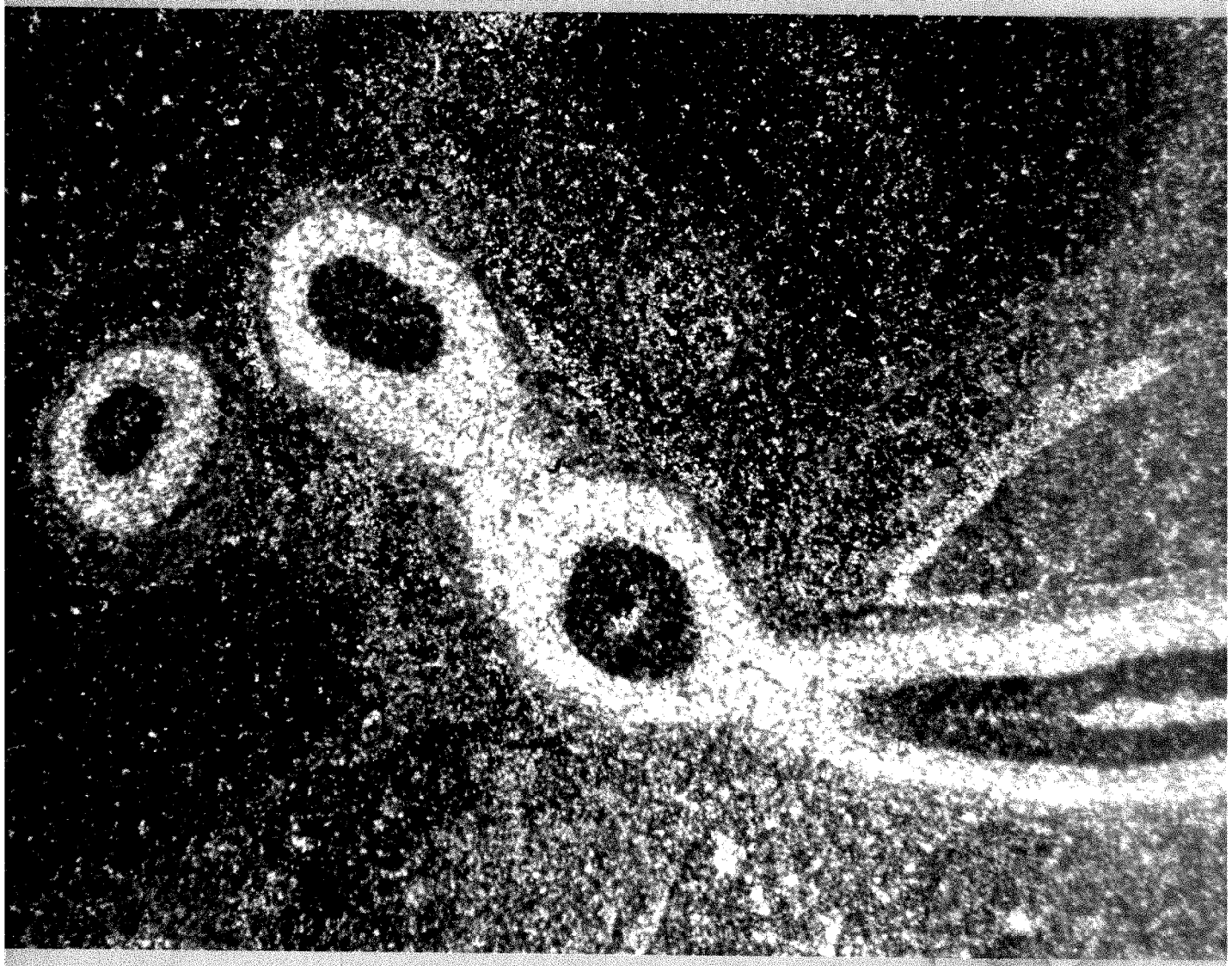
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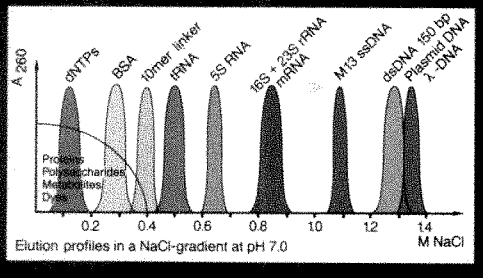
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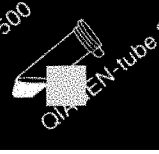
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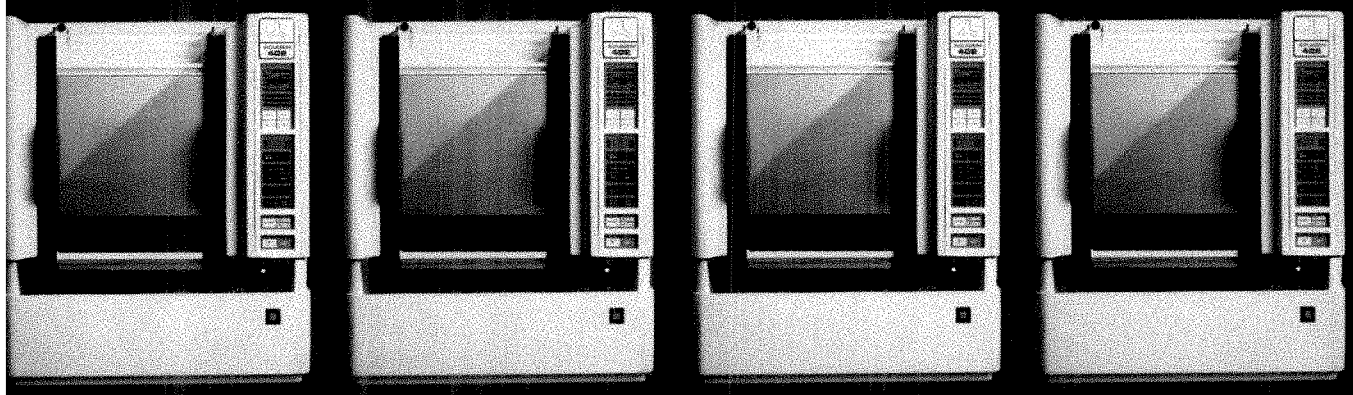


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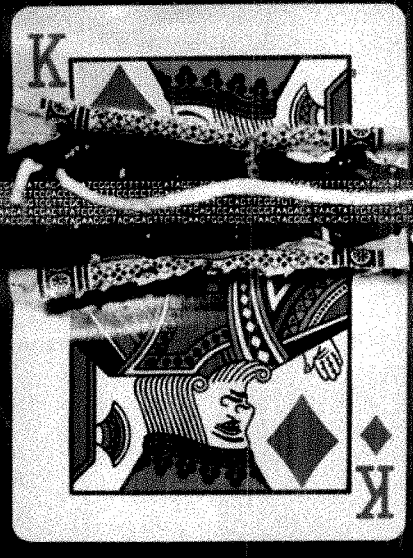
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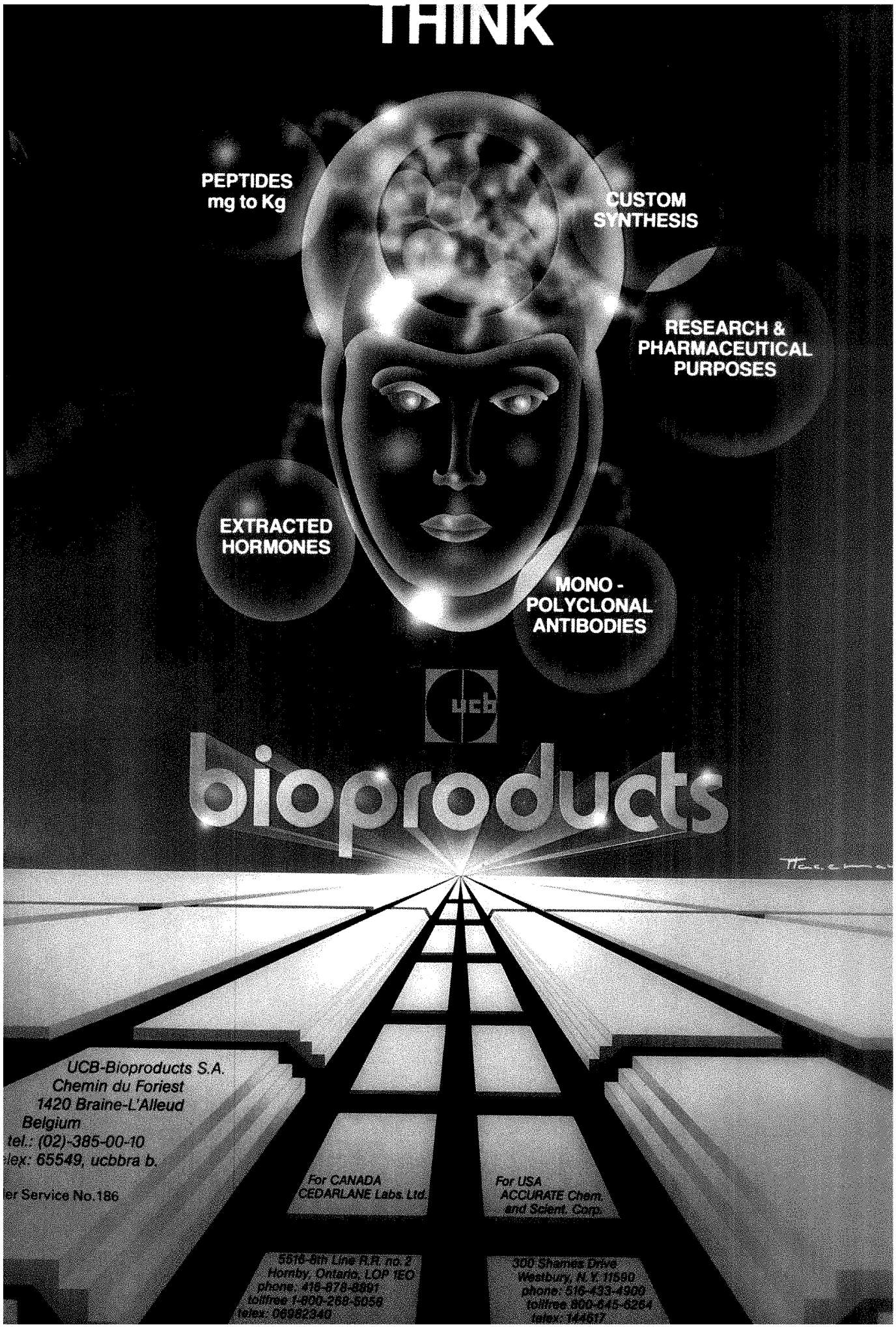
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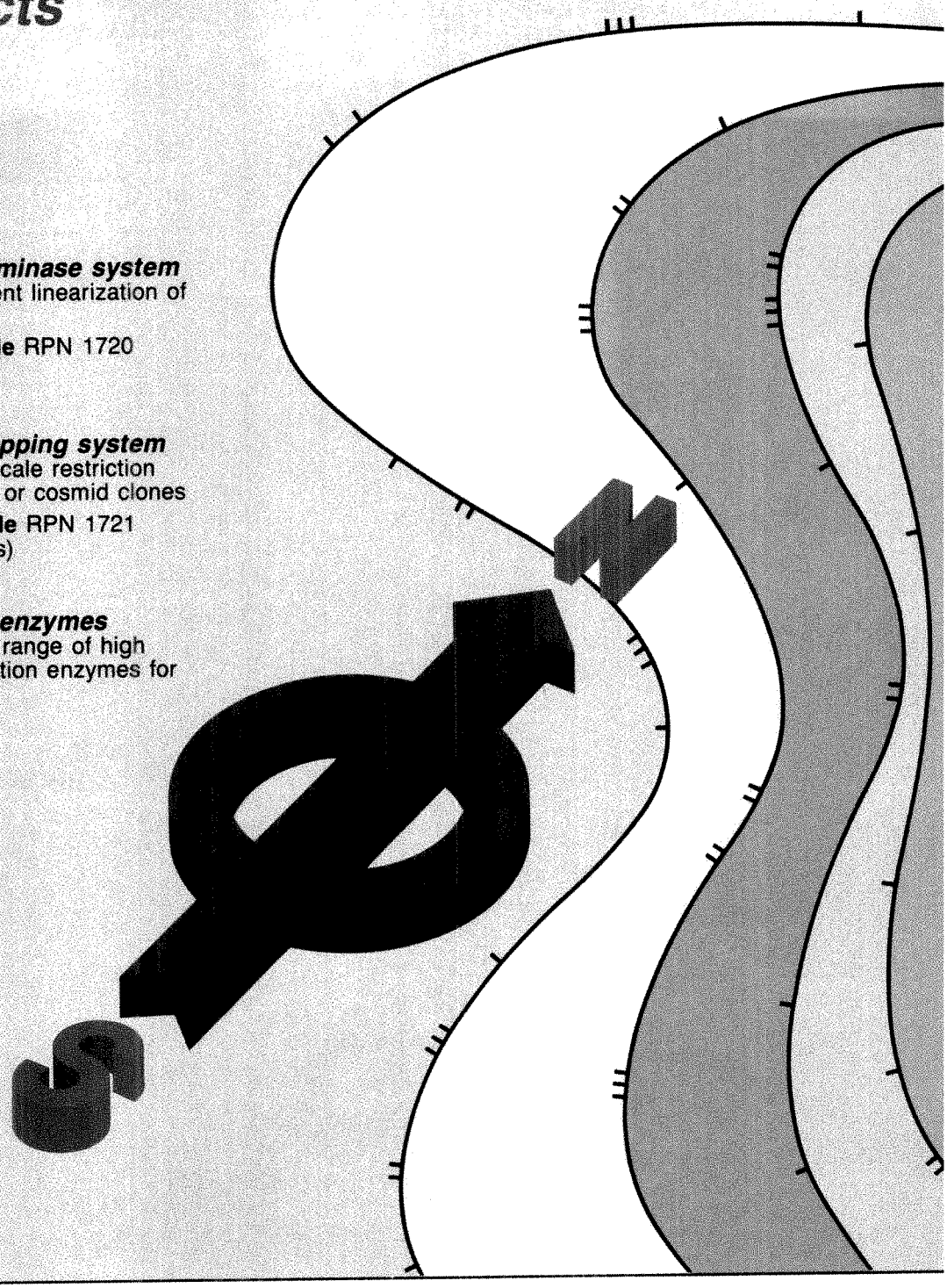


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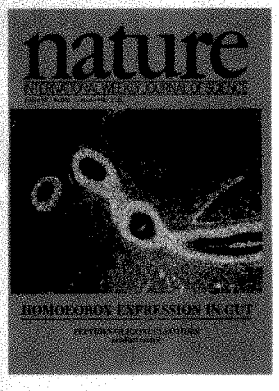
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2 February 1989

Vol. 337 Issue no. 6206

The cover shows the pattern of localization of transgenic homoeoprotein Hox 1.4 in embryonic mouse gut. Mice synthesizing this protein develop a condition similar to congenital megacolon. See page 464.

THIS WEEK

Antarctic ozone

Ozone depletion in the Antarctic atmosphere above 17 km is related to the presence of chlorine monoxide, but balloon measurements made last September suggest that ozone depletion in the lower stratosphere (10–17 km altitude) is directly related to the presence of small cloud particles (0.2 micrometre radius). Page 447.

Nothing to look at

A species of 'eyeless' shrimp that lives close to hydrothermal vents of the Mid-Atlantic Ridge has a 'non-imaging' visual organ that may be a novel eye adapted to detect low-level black-body radiation emitted by the vents' hot water plumes. Pages 458, 460 and 404. In fish and toad retina, the production of mRNA for opsin is regulated by circadian oscillation and in response to light stimulation, page 454.

Strange business

Strange nuclear matter is a hypothetical substance containing approximately equal numbers of up, down and strange quarks. On page 436, ways of making it in the laboratory, and on page 434, accelerator experiments to look for naturally occurring strangeness. And see News and Views, page 405.

Proteins are versatile

Peptidyl-prolyl isomerase, an enzyme involved in protein folding, and cyclophilin, a protein that binds tightly to the immunosuppressive drug cyclosporin, are one and the same. See pages 473, 476 and 407.

A Meaning of Life?

The observation of unexpected anisotropies in the Universe is taken by some to imply a purposeful design . . . page 411.

Under cover

What lies beyond Jupiter's thick cloud cover of ammonia ice crystals? Data from Voyagers 1



and 2 point to the existence of a deeply rooted atmospheric circulation, page 444.

Segmented systems

The use of novel stains reveals regions of the developing brain in chicks with segmental organization like the patterns seen in insect development and perhaps with similar significance in morphogenesis, page 424. Parallel evidence in the mouse is provided by the expression in alternate neuromeres of a zinc-finger gene, page 461.

New phase

A new smectic liquid crystalline phase, in which the molecules are arranged in layers that are free to glide over each other, has, remarkably, both layer and helical order. See page 449.

Nature notes

How long does a *Nature* manuscript take to reach your desk? See page 409. The Guide to Authors faces page 421.

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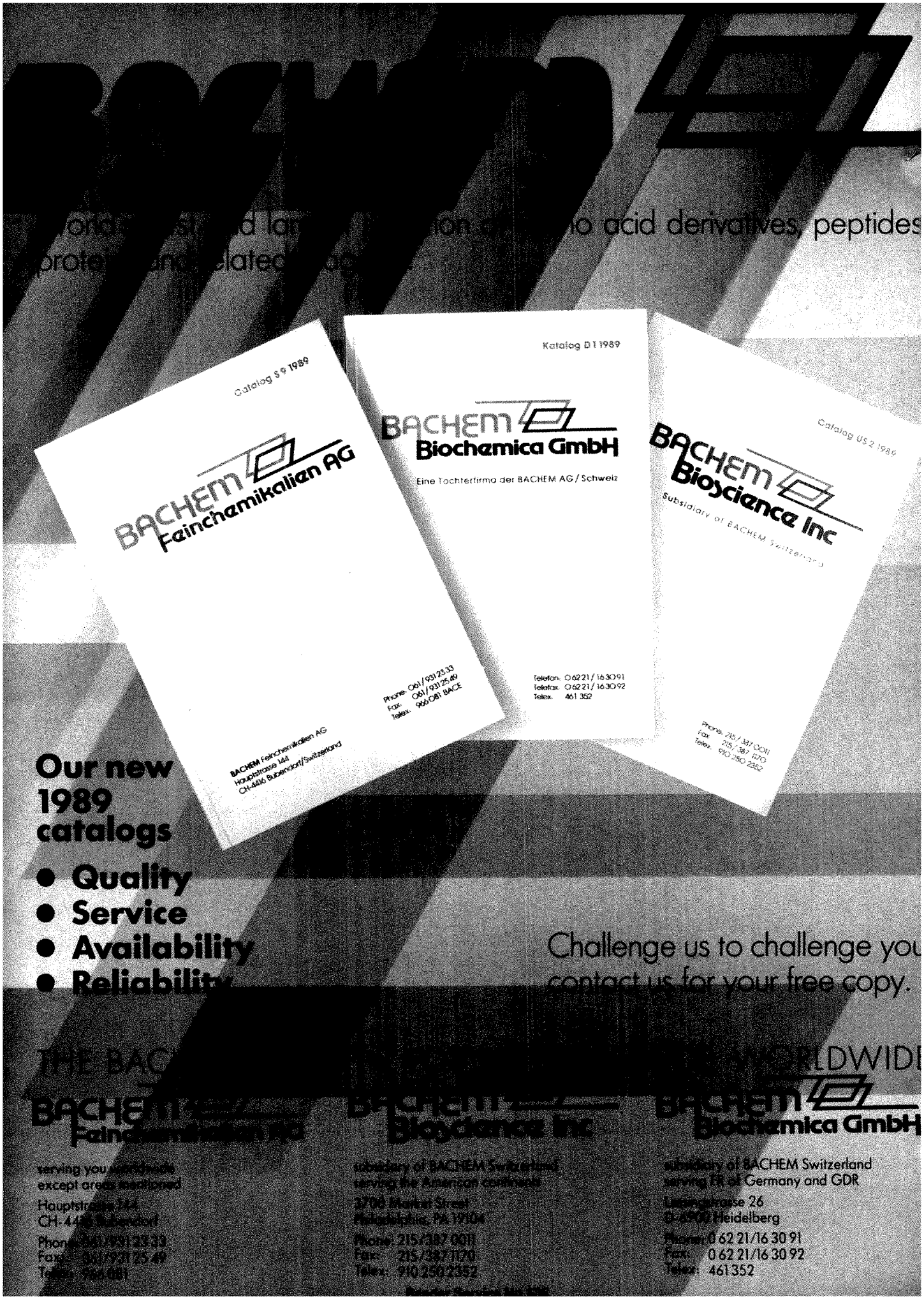
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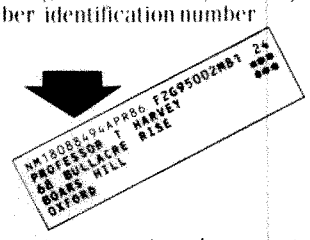
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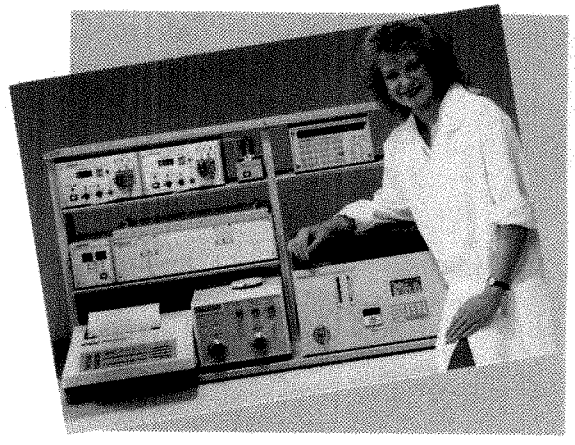
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DNA PROBES

(From the people who gave you Designer Genes™)

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Human Interleukin 1ß

The other major form of IL-1. Probes are available for Exon 5, 6 and 7 of the interleukin 1ß gene.

Human Interleukin 2

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Human Interleukin 3

IL-3 is a growth and differentiation factor for multipotent erythroid and myeloid precursors. Probes are available for Exon 1, 2, 3 and 5 of the IL-3 gene.

Human Interleukin 4

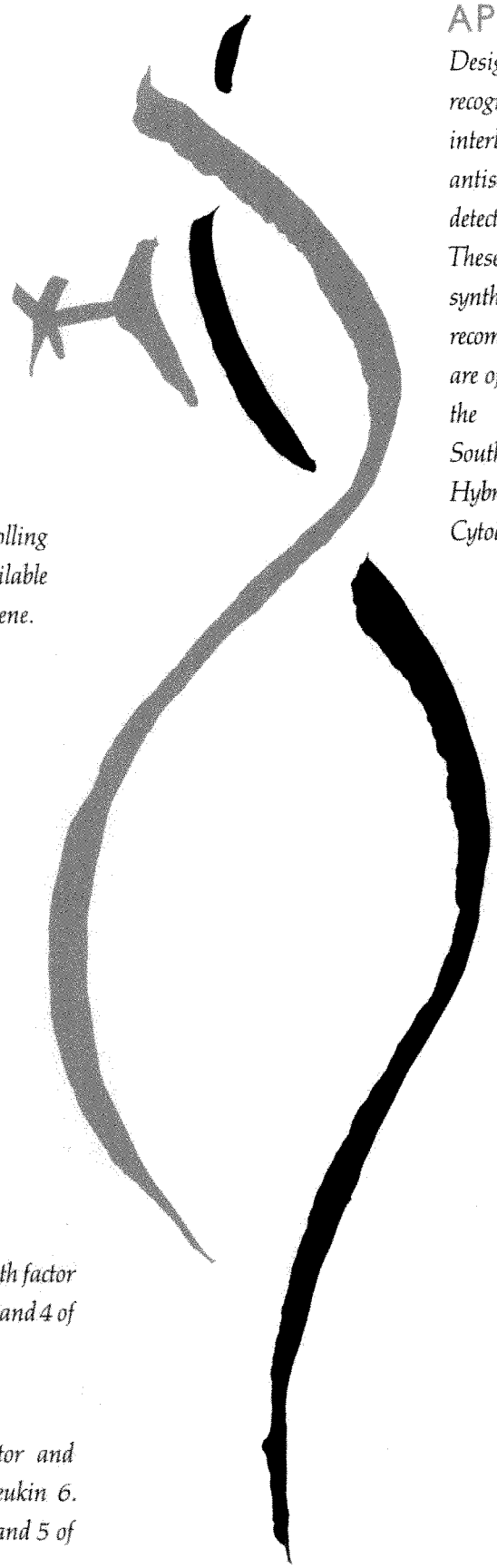
With exon boundaries not yet fully defined for the IL-4 gene, probes have been designed for the 3′ region, mid-region and 5′ region of the IL-4 gene.

Human Interleukin 5

Interleukin 5 is identical to B cell growth factor II. Probes are available for Exon 1, 2, 3 and 4 of the IL-5 gene.

Human Interleukin 6

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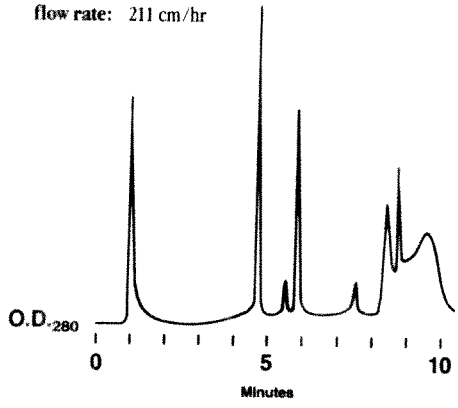
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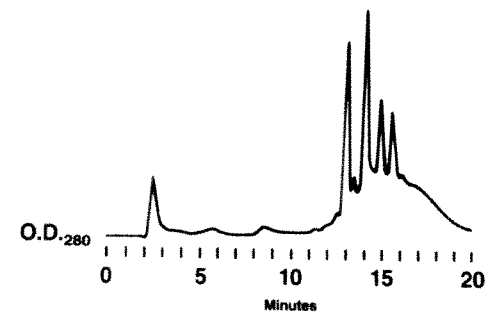
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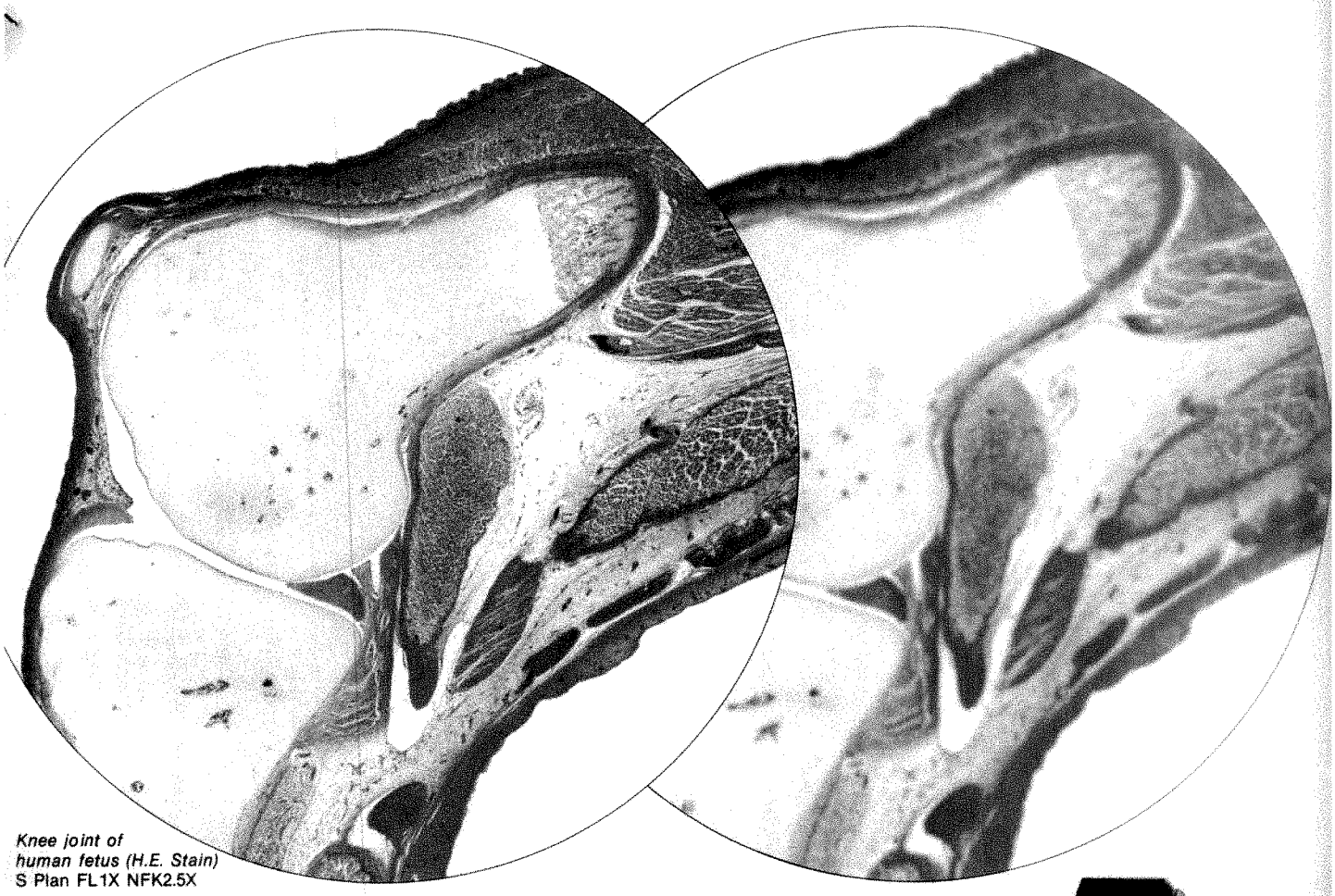
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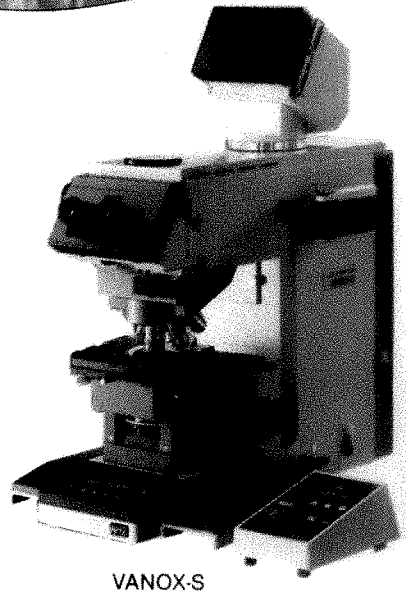
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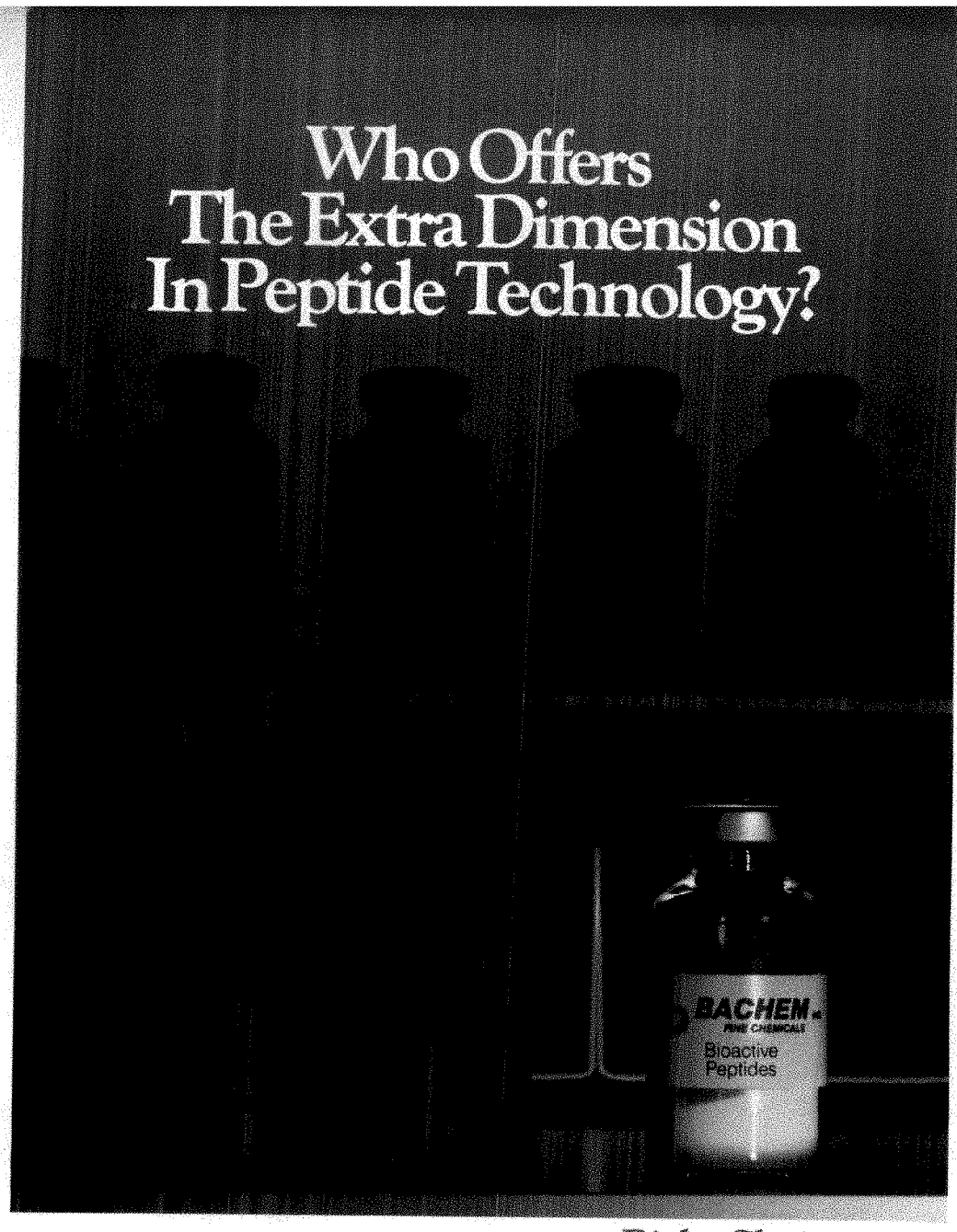


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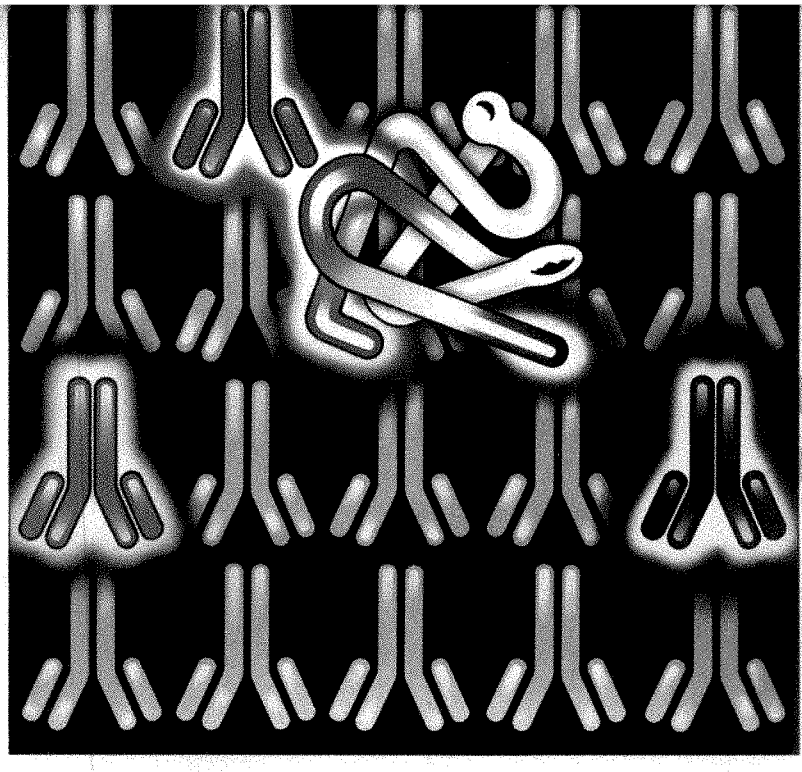
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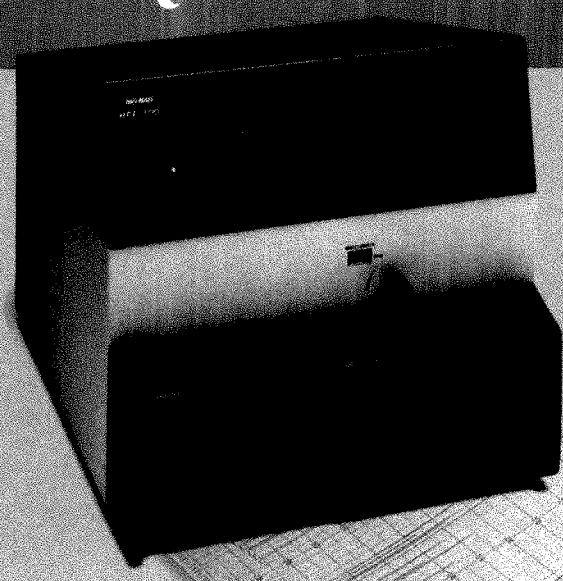
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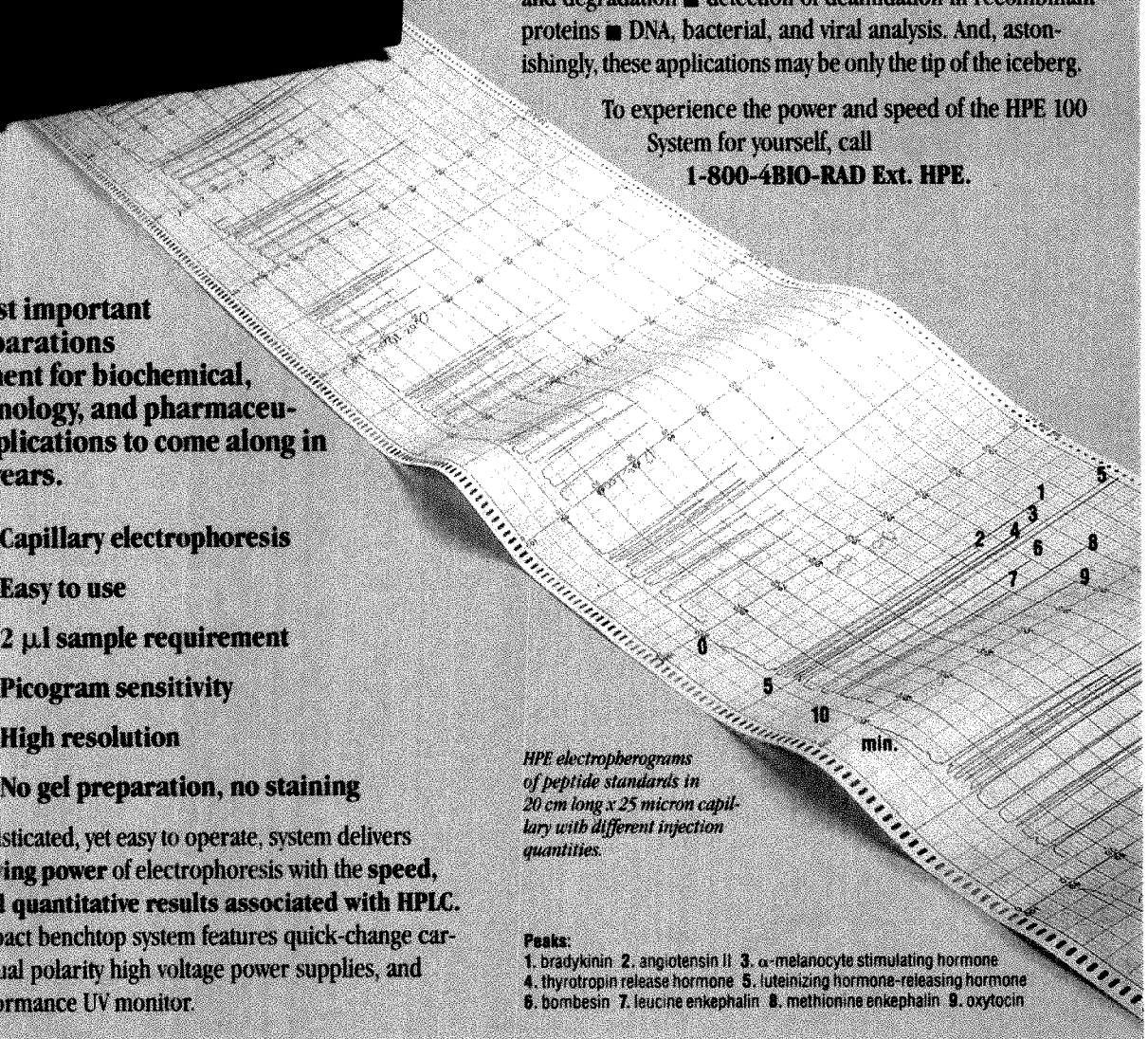
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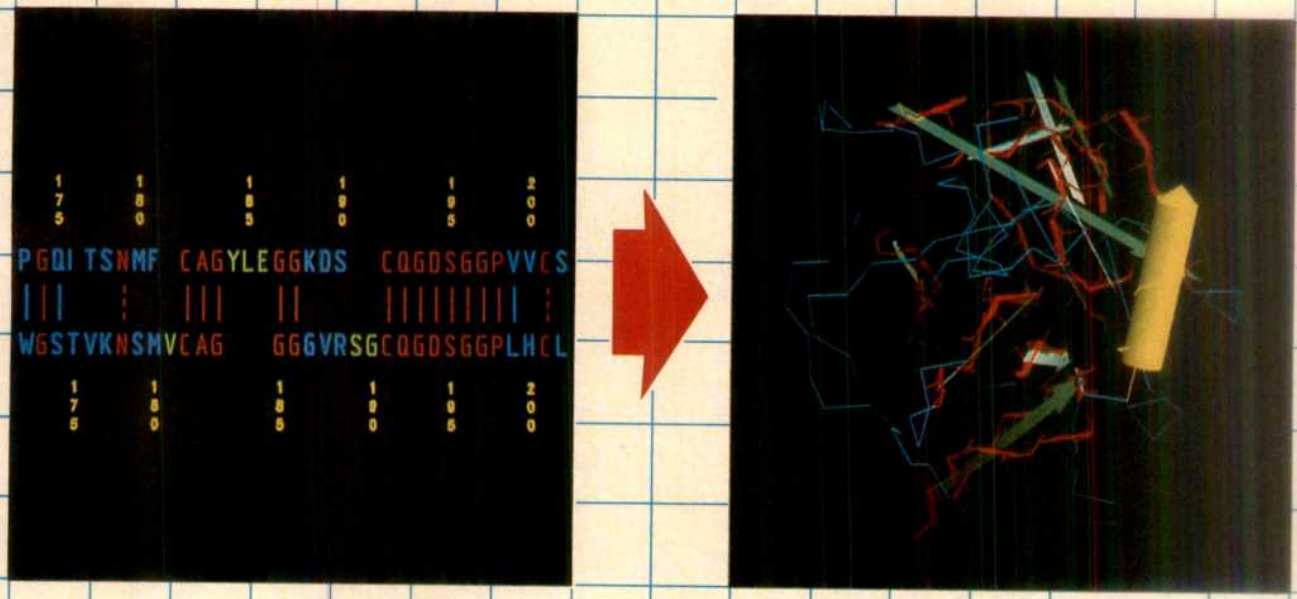
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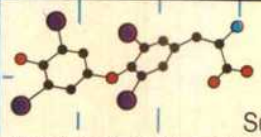
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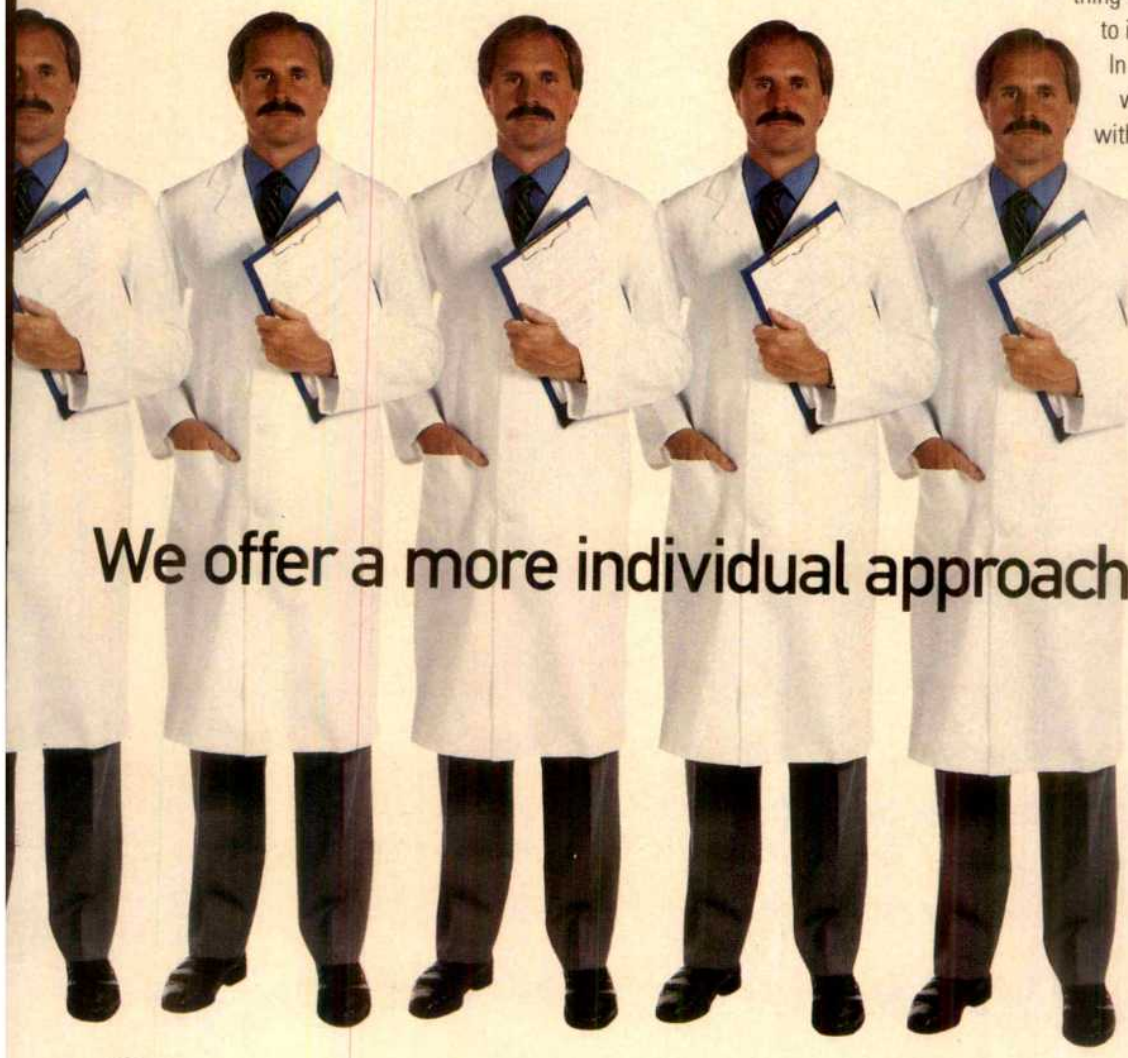
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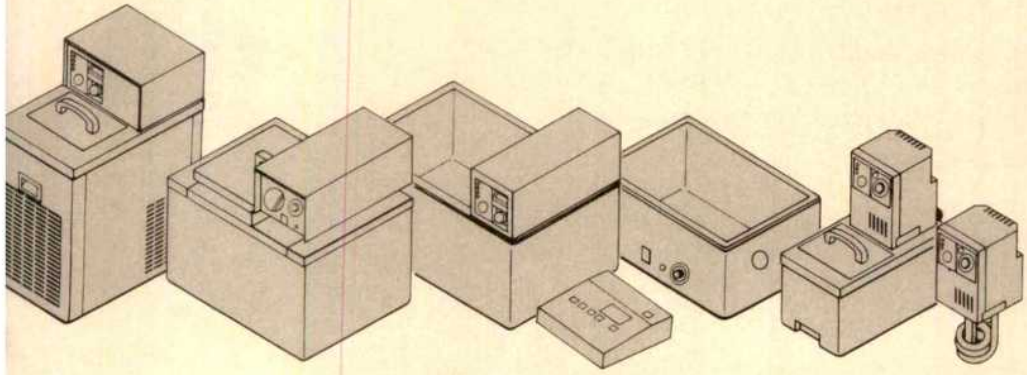
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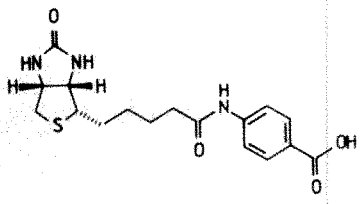
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14408



(+)-N-Biotinyl-4-aminobenzoic acid, B-PABA

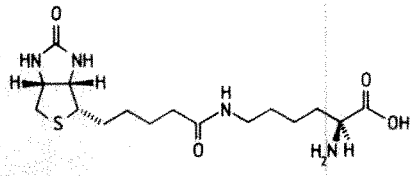
$C_{17}H_{21}N_3O_4S$ M_r 363.44 [6929-40-4]

BioChemika >98% (HPLC)

mp 295–297°; $[\alpha]_{546}^{20}$ +66 ±1°; $[\alpha]_{D}^{20}$ +55 ±1° (c=0.5 in 0.1 N NaOH)
10 mg sFr. 18.– / 50 mg sFr. 75.–

Enzyme substrate used in screening methods for biotinidase deficiency, e.g. in newborns: B. Wolf et al., Clin. Chim. Acta (1983) *131*, 273; N. Engl. J. Med. (1985) *313*, 16; G.S. Hard et al., Clin. Chem. (1984) *30*, 125; H. Wastell et al., Anal. Biochem. (1984) *140*, 69; D.V. Craft et al., Biochemistry (1985) *24*, 2471; K. Hayakawa, J. Oizumi, J. Chromatogr. (1986) *383*, 148; maximum activity of biotinidase for B-PABA is at pH 6–7.5: J. Chauhan, K. Dakshinamurti, J. Biol. Chem. (1986) *261*, 4268.

14409



Nε-Biotinyl-L-lysine, Biocytin

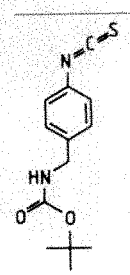
$C_{16}H_{28}N_4O_4S$ M_r 372.49 [576-19-2]

BioChemika >98% (HPLC)

mp 243–245 (dec); $[\alpha]_{546}^{20}$ +69 ±1°; $[\alpha]_{D}^{20}$ +58 ±1° (c=1 in 0.1 N NaOH)
50 mg sFr. 46.– / 250 mg sFr. 185.–

Natural substrate for biotinidase. The fluorometric assay for biotinidase uses 1,2-diacetylbenzene which reacts selectively with lysine allowing its direct determination in mixture with biocytin: H. Ebrahim, K. Dakshinamurti, Anal. Biochem. (1986) *154*, 282; used in transport studies of biotin: J. Chauhan et al., Ann. N.Y. Acad. Sci. (1985) *447*, 386; H.M. Said, R. Redha, Am. J. Physiol. (1987) *252*, G52; preparation of biocytin hydrazide, a selective label for carbohydrates in glycoconjugates: E.A. Bayer et al., Anal. Biochem. (1988) *170*, 271.

15400



4-(BOC-aminomethyl)phenyl isothiocyanate, BAMPITC

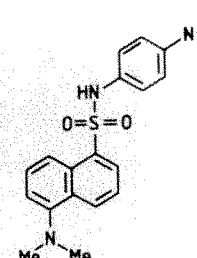
$C_{13}H_{16}N_2O_2S$ M_r 264.35 [89631-74-3]

BioChemika >98% (HPLC)

mp 111–113°; IR-spectrum: in accordance
100 mg sFr. 40.– / 500 mg sFr. 160.–

New protein sequencing reagent with a masked amino group which renders the thiohydantoin – after cleavage of the BOC protection – susceptible to fluorogenic detection, e.g. with OPA. A 2.5 to 10 fold increase in sensitivity over the most commonly used detection methods was observed: J.J. L'Italien, S.B.H. Kent, J. Chromatogr. (1984) *283*, 149; S.B.H. Kent, J.J. L'Italien, US Patent 4,548,904.

39232



4-(5-Dimethylaminonaphthalene-1-sulfonylamino)phenyl isothiocyanate, DNSAPITC

$C_{19}H_{17}N_3O_2S_2$ M_r 383.50 [102417-94-7]

BioChemika >98% (HPLC)

mp 125–128°; IR-spectrum: in accordance; luminescence: passes application test
25 mg sFr. 35.– / 100 mg sFr. 130.–

New, very sensitive Edman-type reagent for manual or automated protein sequencing in the lower picomol range: S.-W. Jin et al., FEBS Lett. (1986) *198*, 150; H. Hirano, B. Wittmann-Liebold, Biol. Chem. Hoppe-Seyler (1986) *367*, 257; J. Salnikow et al. in Methods in Protein Sequence Analysis, K.A. Walsh, ed., Humana, Clifton 1987, p. 247; H. Hirano et al., Eur. J. Biochem. (1987) *170*, 149.

27267

α-Chymotrypsin from bovine pancreas, free of low molecular weight peptide fragments (GPC)

EC 3.24.1.1 M_r ~25 000 [9004-07-3]

BioChemika 65 U/mg; lyophilized

1 U corresponds to the amount of enzyme which hydrolyzes 1 μmol Suc-$(Ala)_2$-Pro-Phe-4-NA per minute at pH 7.8 (25°C)
25 mg sFr. 22.– / 100 mg sFr. 65.–

This α-chymotrypsin is specially purified by ultrafiltration and is free of low molecular weight peptide contaminants and autolysis products.

93608

Trypsin from bovine pancreas, free of low molecular weight peptide fragments (GPC)

E.C. 3.4.21.4 M_r ~23 000 [9002-07-7]

BioChemika ~8000 U/mg; lyophilized

1 U corresponds to the amount of enzyme which increases the absorbance at 253 nm by 0.001 per minute at pH 7.6 and 25°C (N-benzoyl-L-arginine ethyl ester, FLUKA 12880, as substrate)
25 mg sFr. 18.– / 100 mg sFr. 60.–

This trypsin is specially purified by ultrafiltration and is free of low molecular weight peptide contaminants and autolysis products.

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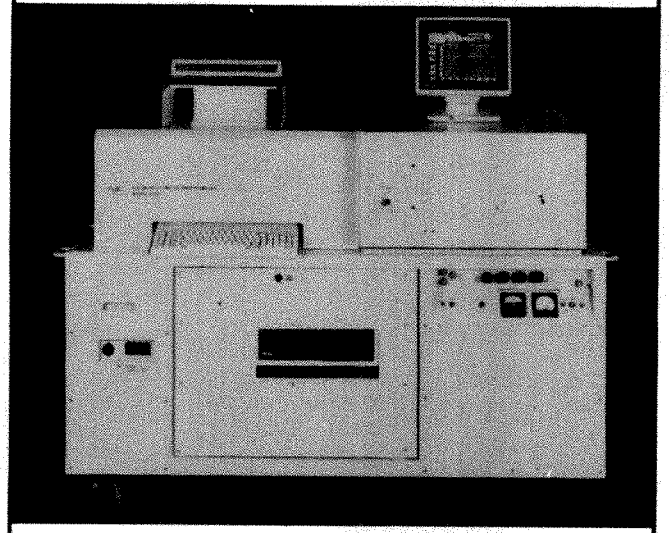
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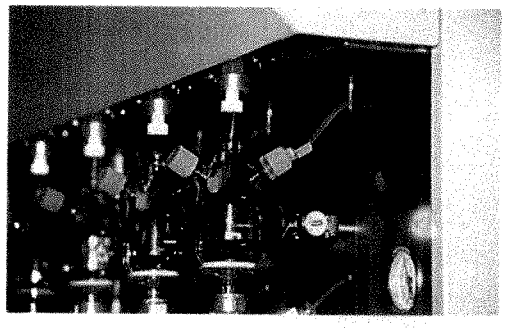
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nature

London kidney exchange in trouble

British transplant physicians are in hot water for allegedly transplanting kidneys obtained by purchase. But buying kidneys may not always be wrong.

In the design of mammals, there is a useful measure of redundancy: some organs, the kidney conspicuously, appear in pairs, the redundancy being a safeguard against the stresses of long life: if one of a person's kidneys should fail, the other may be sufficient. Kidney transplants are a further safeguard. Most transplanted kidneys derive from cadavers, but because a person is not fatally or even severely damaged by the removal of one kidney, some are from living people, usually close relatives of the recipient. Nobody disputes the value of the procedure (which in Britain is believed to save £50,000 for each renal patient taken off dialysis machines). Worldwide, some tens of thousands of transplant operations are carried out each year, mostly in developed countries. (Just under 1,700 cadaver kidneys were transplanted last year in Britain alone.) The success rate is high (about 80 per cent one year after the operation, half as much ten years later).

Physiological redundancy has now also, it seems, stimulated a trade in human kidneys. For the past two weeks, London newspapers have been in pursuit of what they allege to be an international racket. There is proof that Turkish newspapers carry advertisements from would-be donors. It is alleged that a two-legged conspiracy based in Istanbul and London arranges for donors to be shipped to London for operations and, afterwards, to be paid. Some of the subsequent transplant operations are said to be carried out at London hospitals in the private sector. That makes the issue especially piquant on the eve of the publication of the government's proposals for the reorganization of its National Health Service, known to include suggestions that major hospitals in the public sector may opt for autonomy, selling medical services to the public and private sectors alike.

Legislation

If (as is probable) there is a trade in kidneys from living donors, there will also soon be vigorous demands in Britain that it should be made illegal. As things are, physicians are enjoined by professional guidelines not to take part in operations in which organs from living donors have been sold, although close relatives of a person with kidney failure may donate a kidney out of altruism. There is also a Council of Europe declaration that would outlaw the practice of transplanting organs obtained by purchase from living donors — if the declaration had legal force. But the question of whether there are circumstances in which a person may be allowed to sell a kidney of his or her own for cash is more complicated than it may seem.

Operationally, the underlying principles are clear, even simple. Physicians with patients in need of a kidney transplant naturally owe their first duty to them, but are not thereby ethically absolved from the responsibility of enquiring into the means by which kidneys for transplant are obtained. In part to protect the zealous transplant physicians from their proper zeal, it is standard practice when transplant organs are obtained from patients who have died in intensive care that an independent physician is responsible for deciding when the putative source is brain-dead. The same principles should apply with the transfer of organs from one living person to another. It should be for a physician independent of the intended recipient to shoulder ethical responsibility for the interests of the would-be donor.

Naturally, this second physician's chief responsibility (apart from seeking contra-indications to the proposed operation) must be to ensure that the intending donor is fully informed of the consequences, which are not as innocuous as the notion that one kidney is redundant would suggest. In normal people, kidney function becomes less efficient in middle life, and then progressively declines. However healthy a young donor may appear, losing a kidney must increase the risk of illness or even death in the second half of the normal lifespan. It is crucial to an understanding of what has been happening in London to know whether the Turkish donors so far tracked down were made fully aware of these extra risks. In principle, they might have been partly compensated by some form of health insurance, but there has so far been no suggestion of such arrangements. Indeed, the physicians most obviously concerned in the protection of the donors' interests have been singularly uncommunicative so far, yet they are those who shoulder the chief ethical responsibility.

With these provisos, there are two reasons why the practice of rewarding kidney donors even with sums of money large enough to provide an incentive should not be unthinkingly banned. First, there may be circumstances in which the balance of a potential donor's interests suggest that cash now justifies the risk of ill-health later. The simplest version of the hippocratic doctrine that no physician shall ever act so as to hazard a patient's health is easily challenged. What if the intending donor needs cash to care properly for a handicapped child, for example? Is the altruism that justifies the transfer of organs between relatives never transferrable to third parties? Even when there will be two beneficiaries (the recipient of the kidney and the object of the displaced altruism), not just one? It goes without saying that physicians will not win the freedom to use this or other possible shadings of hippocratic principles unless they are not merely willing, but eager, to describe and explain their conduct towards donors publicly — and to demonstrate that their own rewards are seemly. No doubt the medical profession's regulators will act with appropriate severity if these explanations do not emerge from the inquiries now under way in London.

The second reason why it is unwise to attempt to ban these practices is that the law is unlikely to be effective. Physicians where medical practice is relatively advanced inevitably attract patients from elsewhere. But the demand for kidney transplants is understandably so clamant that the trade (if there is one) will, if banned, shift to less squeamish places where standards of practice and (usually) ethics are high. Can that be to the greater good? Much the better way of blunting the problem is to ensure that the supply of organs for transplant is sufficient. In Britain, the National Transplantation Tissue Service, which operates within the public health service, is now able to meet only two-thirds of the demand for kidneys. British private-sector medicine derives a few dozen kidneys a year from dwindling unmatched supplies from the United States, but otherwise relies on living donors. Yet the shortfall is not so great, and might be made good by better international arrangements for collecting, matching and delivering kidneys to where people need them. That, not legislation, is the way out of this dilemma. □

Japan's science budget expands

- Spending reflects role as world power
- Massive budgets for space and nuclear power
- More money for superconductors and AIDS

Tokyo

JAPAN's cabinet last week finally approved an expansionary ¥60.41 million million budget for fiscal year 1989. The budget is late, delayed by battles over the introduction of a new sales tax and by the resignation of three cabinet members who have admitted involvement in the Recruit stock scandal. But even with public confidence in the government at a new low, the economy is untroubled; a predicted 4 per cent growth rate lies behind the decision to let the budget rise 6.6 per cent over 1988.

Big increases in defence, overseas development assistance and student exchanges mark Japan's increasing awareness of its role as a world power. Science and technology budgets are also characterized by the appearance of new programmes to aid in the internationalization of Japan's research system, in line with the Council for Science and Technology's guidelines to emphasize international contributions and creative research and development in 1989.

All the international programmes are still tiny in financial terms, and despite the guidelines many had to be rescued at the very last minute from a hard-hearted Ministry of Finance. Even the Human Frontiers Science Program had to struggle for survival but it is finally under way, more than three years after first being proposed, with a budget of ¥2,400 million ($19 million) split between the Science and Technology Agency (STA) and the Ministry of International Trade and Industry (MITI). The programme is intended to promote basic biological research through international cooperation (see *Nature* **334**, 281; 1988) and will have its headquarters in Europe. London is the favoured location but Paris, Brussels or Heidelberg are still possibilities.

Both STA and MITI will also set up new organizations to look after foreigners coming to work at their research institutes. Problems of language, culture and accommodation have so far been left to each institute to solve (or abandon) in its own way. Increasing numbers of foreigners should be on their way to Japan, with the budget granting extra fellowships for scientists from the seven summit nations at STA and the Ministry of Education, Science and Culture (MESC). But filling those places already available remains a problem (see *Nature* **335**, 287; 22 September 1988).

Within STA, budget increases go to both the International Frontier Research System and Exploratory Research for Advanced Technology (ERATO) programmes. Both have been specially designed to break down the hierarchical structure of Japan's research laboratories and to involve foreign researchers. B the really massive budgets go to spac where development of the H-II rocket under way, to ocean research, where t Shinkai 6500 submersible has just be launched (see page xxx), and to nucle power, where a special account provid large sums for studies of nuclear pow plant safety. Opposition to nuclear ener is now increasing in Japan at a runaw pace. Earth science expenditure al booms with the launch of the Mari Observation satellite (MOS-1b) due f 1989, followed by the Earth Resourc Satellite ERS-1 in 1991.

Expenditure at TRISTAN comes dow (but running time goes up) as constructio of the electron–positron collider is com pleted. Spending is also down on ocea sciences as the construction of the ne oceanographic research vessel *Haku maru* nears completion. Big increases a given to nuclear fusion research, to co struct the world's biggest helical fusio device near Nagoya, and in space researc to prepare for the 1990 launch of MUSES 1, a test satellite which will attempt t swing-by the Moon.

Both MITI and the Environmer Agency join in international concern ove global environmental problems, MITI wi a initial 'concept' for a major project an the Environment Agency with a budget t develop an ozone monitoring device to fl on the Advanced Earth Observing Sate lite 1993. The Environment Agency als receives funds to monitor acid rain and t put on showcase international enviro ment conference in Tokyo.

International trends are also reflecte in the predicted big budgets for researc on superconductors, both high-tempera ture and conventional, at STA, MIT and MESC, and in increases for AID research by the Ministry of Health an Welfare (see *Nature* **335**, 194; 15 Septem ber 1988) even though the number o AIDS cases remains very small.

Both MITI and the Ministry of Trans port are also looking at projects to tunne beneath Tokyo at depths greater than 5 metres, beyond where landowners' right end. Land prices are now so high tha Tokyo could (in theory) be mortgaged fo sufficient money to buy all the private lan in the United States plus all the companie quoted on the US stock exchange (th transaction is not practical, althoug Japanese politicians like to joke abou buying something smaller, California fo example). The Ministry of Transport i interested in deep subways; MITI has a more futuristic plan to put factories i caves deep underground where even foreigners may be able to afford the rent.

Alun Anderson

Details of the Japanese science budget

	1989 ¥000 million	% change from 1988
Human Frontier Science Program	2.4	—
Science and Technology Agency		
Space	108.9	+10.6
Nuclear energy	279.9	+3.1
Ocean research	10.5	+11.1
Earth sciences	30.4	+72.0
ERATO	4.5	+18.4
International Frontiers	1.8	+11.8
Ministry of Education, Science and Technology		
Research grants	51.8	+6.6
Fellowships	1.9	+27.1
Accelerator physics	15.5	−6.1
Space science	21.0	+6.1
Nuclear fusion	9.2	+21.4
Marine science	2.9	−46.7
Ministry of International Trade and Industry		
New Energy and Industrial Technology Development Organization	7.2	+64.1
Basic technologies for future industries	6.8	+7.2
Large-scale industrial projects	14.0	+3.5
Sunshine project	37.5	+3.9
Moonlight project	10.5	+7.3
Fifth-generation computer	6.4	+12.5

UK universities request £183m

London

BRITISH university vice-chancellors last week asked the government for an extra £183 million over the next two years to help recruit and retain high quality staff and put an end to the pay dispute with academics, now into its fourth week.

The Committee of Vice-Chancellors and Principals (CVCP), asked for £64 million for 1989–90 and £119 million for 1990–91. This would finance a 6 per cent pay rise for all academic staff and leave some extra money to raise salaries in areas where there is a staff shortage and to reward staff of outstanding merit.

Academic salaries have fallen by more than 20 per cent relative to average earnings since 1979, said Richmond, in a letter to Kenneth Baker, the Secretary of State for Education. The CVCP has already offered staff a 3.5 per cent pay increase for this year but it recognizes that this is inadequate. If this offer cannot be improved, salaries will fall by a further 5–6 per cent in 1989, says Richmond, and to pay competitive salaries, numbers will have to be cut. The Association of University Teachers (AUT) has criticized the CVCP request, pointing out that even if this money is forthcoming, pay will still be 20 per cent behind average earnings relative to 1979 levels. It demands a substantial increase in basic pay levels, not just rewards to certain groups and individuals.

A survey for the CVCP has shown that 36 per cent of professorial posts and 19 per cent of lectureships were vacant last year. Recruitment of professors was particularly difficult in mathematics, accountancy, electrical engineering and computing. For lecturers, it was also difficult in general science, engineering and business studies.

The CVCP wants to add three discretionary salary points to the top of the lecturer pay scale to avoid having to promote to senior lecturer, which it says is unnecessarily expensive.

The committee also asked for £28 million for payment of overheads on research contracts. Because earnings from research councils and charities have increased, the University Grants Committee can no longer comply with its own instruction that universities should receive 40 per cent in overheads on research contracts, said Richmond. It also wants to increase technicians' salaries. In 1986, they were paid 18 per cent below market rates, and by April this shortfall will be 27 per cent.

The AUT boycott continues to disrupt examinations throughout the country; mid-sessional examinations are being cancelled and papers for summer examinations are not being set.

Christine McGourty

West German biochemistry institute set ablaze by "viruses"

Munich

MOLECULAR biologists in West Germany have been sleeping uneasily since 2 January, when arsonists broke into a laboratory in Darmstadt and started a fire that caused damage estimated at DM1.5 million.

Authorities say that the early-morning attack on the biochemistry institute of the Technische Hochschule in Darmstadt was the work of an 'autonomous' group thought to be loosely affiliated with the terrorist Red Army Faction. Because of the possible terrorist connection, the case is being investigated by the federal prosecutor-general. The only clue to the identity of the criminals is a letter, postmarked in Frankfurt, that was sent to news organizations.

In the letter, the "angry viruses" (*zornige Viren*) — a previously unknown group — take responsibility for the attack. The letter, written in a convoluted style, calls genetic engineering a tool of Western imperialism used to exploit the developing world. The use of such techniques in agriculture, says the letter, is directly related to the "programmed starvation" of subsistence farmers. Human genetics and reproductive technologies are the "definitive attempt" to subject the fertility of women to masculine exploitation and control.

The director of the biochemistry institute, H. G. Gassen, is singled out in the letter as a "clever propagandist of genetic engineering". Gassen had become known locally as the initiator of a university–industry consortium for pharmaceutical research. The letter accuses Gassen of trying to increase public acceptance of genetic engineering by acknowledging specific criticisms but defending the research in general.

The attack is the latest in a series of assaults on research institutes and private companies that use molecular biology techniques. Earlier attacks have been reported in West Berlin, Cologne, Braunschweig, Münster, Hannover and Heidelberg.

The West German press and the public have expressed strong reservations about genetic engineering in recent years. West German police have alerted other private and public research institutes of the danger of a possible repeat attack.

Gassen, whose work involves the development of synthetic enzymes for diagnostic purposes, says he will not let himself be intimidated by the attackers. "That's just what they want — for us to perform our research in secret behind locked doors. We refuse to play their game." The fires were set in four places on the sixth floor of the biochemistry institute using bags of gasoline hooked to a welding kit and a timing device. Gassen's isotope laboratory was left coated with soot and will be unusable for at least three months.

The fire would have endangered "ten years of research", says Gassen, but it was extinguished by the fire department before it could do more damage.

Steven Dickman

Seasonal greetings at high dilution



THE Department of Pharmacology at the Free University of Amsterdam follows the practice of commissioning a solsticial greetings card from its two newest members. This year's card is reproduced above. □

New warmth via research scheme

Tokyo

THE first meeting last month of the Joint High-Level Advisory Panel set up under the new US–Japan Science and Technology Agreement focused on problems of 'comparable access to common base technology' in Tokyo. But Japanese representatives also expressed interest in participation in research on global environmental change, a new area for Japan.

According to officials at Japan's Foreign Ministry, Prime Minister Noboru Takeshita has been advised that Japanese participation in research on the greenhouse effect and acid rain would be well received in the United States. Takeshita intends to host a conference on global environmental problems in Tokyo this year and may propose a joint US–Japan research programme when he visits Washington next month.

The fourteen members of the advisory panel include Deborah Wince-Smith, assistant director of the US Office of Science and Technology Policy, Mary Good, chair of the US National Science Board, and Robert White, president of the US National Academy of Engineering. Among the Japanese members are Michio Okamoto, former president of Kyoto University, Saburo Nagakura, president of the Graduate University of Advanced Studies, and Minoru Oda, president of the Institute of Physical and Chemical Research. Wince-Smith said that discussions had been "free" and "very positive" and that the group did not try to "revisit" the issues that made negotiation of the US–Japan agreement long and difficult. But several Japanese participants noted that the problem of "comparable access" was not easy to solve quickly.

Both nations would like to see equal access to each other's research at the 'generic' or 'pre-competitive' stage, that comes before 'applied research' generates actual products. The problem is that whereas US base technology is often developed in government laboratories, to which foreign researchers have access, much Japanese technology is developed in private companies closed to outsiders. The US government has considerable leverage even over research performed in private laboratories, as much of it is paid for by the Department of Defense funds. The same is not true in Japan.

Japanese panel members stressed that progress towards putting foreign researchers in company laboratories is likely to be slow, and that much discussion is needed. Some members believe the quickest way to increase joint access to basic research would be to strengthen Japan's university system. **Alun Anderson**

Foreign critics hit a nerve in self-conscious West Germany

Munich

FOREIGN researchers' eloquent criticisms of academic life and society have caused a stir in West Germany, a country unusually concerned about what foreigners think of it. The criticisms appear to have hit a raw nerve throughout the country, provoking a response of shame and self-analysis. West Germany has had to cope with a rising number of foreign workers and ethnic German 'returnees' from Eastern Europe.

The widely reported comments appear in a report (*Through the eyes of others: experiences of foreign researchers in Germany*) issued by the respected Alexander von Humboldt Foundation. The non-profit foundation brings hundreds of researchers to West Germany every year for periods up to two years. It has supported more than 12,000 foreign researchers in the past 35 years.

The negative experiences described in the report stand in sharp contrast to the generally glowing reviews received in the past. This may in part be explained by the fact that Humboldt fellows have only recently been required to fill out questionnaires about their stay in Germany. Voluntary contributions formed the basis of earlier reports issued in 1965 and in 1977.

Although the overwhelming majority of researchers felt they profited from their stay in West Germany, the criticisms attracted the most attention. Most disturbing to West Germans was the increasing xenophobia and insensitivity that the researchers encountered in West German laboratories, libraries and on the streets.

One frequently quoted account was by a black South African scholar who first came to West Germany in 1958. He wrote that in his first three-year visit he had been openly confronted with racism only once and had struck up friendships with West Germans on the street and in the labour unions where he sometimes worked, leading him to consider West Germany his "second homeland". Twenty years later, he felt "wounded" by "harsh, unpremeditated words flung at him" in the streets.

Although his experiences are more extreme than many of those described by foreign researchers, the image of "material riches" accompanied by "emotional poverty" was a common theme in their reports.

In addition to its message for West German society at large, the report included criticisms of everyday university life. Comments such as "more confrontation than cooperation" and criticisms of the hierarchical organization were commonplace, especially from West European or North American researchers. One researcher wrote that "German professors are the last German kings".

Despite the criticisms, Heinrich Pfeiffer, general secretary of the Humboldt Foundation, said that in the past eight years the foundation has been "overrun with applications" from all over the world. It is at present planning a drive to raise money so that it can offer a hundred new fellowships to researchers from China and the Soviet Union. There have been growing numbers of applications to the foundation from both countries in recent years. **Steven Dickman**

Harvard group to teach Soviet diplomats

Boston

THE Soviet Ministry of Foreign Affairs has announced that it will accept an offer by the Harvard Negotiation Project to conduct a five-day 'negotiation workshop' for mid-career Soviet diplomats in Moscow this spring.

Although bilateral cooperative efforts have lately been flourishing between the United States and the Soviet Union, negotiation skills of diplomats have not kept pace with the improved climate, according to the Negotiation Project's director Roger Fisher.

Fisher, a professor at Harvard Law School, says the new arrangement represents "a crucial, early step in the development of a needed skill", namely to learn "how to handle differences" between the two nations through diplomatic channels. The negotiation workshop will be held at the Soviet Ministry's Diplomatic Academy in Moscow, which provides advanced training for experienced Soviet diplomats.

Among Fisher's suggestions for alternative processes are "pre-negotiations" where parties sit down together to reach a mutual understanding of the scope and quality of the outcome desired through the negotiation process. Fisher calls international conflict "a growth industry", and says that improving conflict resolution procedures is necessary "in order to make the civilized world work". Now that the Soviets have agreed to a workshop, the Negotiation Project wants to persuade the State Department of the United States to accept a similar arrangement. So far, the State Department has not accepted the offer.

Seth Shulman

European molecular biology laboratory in search of a director

London

THE withdrawal of the only candidate for one of Europe's top scientific posts has created a considerable problem for the governing body of the European Molecular Biology Laboratory (EMBL) in Heidelberg. The most likely outcome of a meeting of EMBL's council on 6 February is that the present director, Lennart Philipson, will be asked to stay on beyond April 1990, when he was to have been replaced.

The hunt for a successor to Philipson, who has been director since 1982, has been under way for two years. A search committee chaired by the council's chairman, Professor Jan Drenth, scoured Europe for suitable candidates and identified a handful. But only one of them, Professor Tom Blundell of the Crystallography Department, Birkbeck College, London was interested. He withdrew his candidature two weeks ago after some months of negotiation.

At a council meeting last December, it became clear that a small number of the 14 countries that provide EMBL's funds were opposed to Blundell's appointment, and a decision was postponed until next week's special meeting There has been little or no weakening of the opposition and it seems unlikely that the ten votes necessary for the appointment would have been cast at the meeting.

One reason given for opposing the appointment, particularly by the French delegation, was that EMBL should not have a structural biologist as its director. But there was also opposition, much of it from within EMBL, to Blundell's condition that he should be allowed to continue his personal research, even though his proposal was for a modest group of 10 people.

Blundell says he had been greatly looking forward to the challenge of the job, and particularly the task of sorting out the central facilities of EMBL, including its out-stations and the biocomputing laboratory, whose work falls within his own expertise. Instead, he now hopes for rapid progress in his negotiations with the Imperial Cancer Research Fund for a substantial programme of collaborative research.

EMBL's council is faced with the choice of restarting its search for a successor to Philipson or extending his stay. Drenth feels that it would now be in the best interests of the laboratory if Philipson would stay on. And Philipson is believed to be willing to stay if certain conditions are met.

Peter Newmark

BMA scorns loans

London

THE British Medical Association (BMA) this week criticized the government's plans to introduce a loans system for students to supplement the current grants system, saying that students would be deterred from entering the medical profession, especially those from poorer families. The profession is disturbed at the prospect that its future stock will be drawn from those who can afford to pay for a medical education rather than those best suited by their ability to benefit from it, says the association, in a letter to the Department of Education and Science.

Surveys reveal considerable disillusionment among young doctors about careers in medicine, says the BMA. Of those qualifying in 1981, 46 per cent expressed regret at having entered medicine, compared to 28 per cent in 1976. If young doctors are also faced with large outstanding loans from their undergraduate days, this disillusionment will only get worse. The association estimates that by 2006 a junior doctor could have a total loan to repay of up to 35 per cent of his or her first year's income.

The BMA urges the government to reconsider its plans for a loans scheme and instead to restore the recent loss in real value of the grant. Christine McGourty

Arianespace order

Paris & Tokyo

THE night of 27 January was a busy one on launch pads around the world. At 01.21 GMT, Arianespace, the consortium operating European space launches, put an Intelsat V communications satellite into geostationary orbit. Just a few hours earlier, on the other side of the globe, the Japanese National Space Development Agency successfully launched the TR-1(2) prototype for its H-II rocket.

The European launch from Kourou in French Guiana confirmed Arianespace's lead in the commercial satellite launch market. With an order book for 36 launches, nine of them scheduled for this year, Arianespace hopes to repeat last year's turnover of $520 million.

In February, Arianespace will sign an order for 50 Ariane-4 launchers, worth more than $4,500 million, and intended to be the company's workhorses until 1999. Japan hopes one day to take a slice of that market. But it will be at least 1992 before the H-II rocket is complete, and commercial launches are not expected to begin until the late 1990s. By then, H-II's 4-tonne payload may look small compared with that of planned new Ariane launchers.

Peter Coles & Alun Anderson

New chairman

Munich

THE West German Wissenschaftsrat (science council) chose Dieter Simon as its new chairman at a meeting in West Berlin on 27 January. Simon, a law scholar and director of the Max Planck Institute for European legal history in Frankfurt, replaces physician Kurt Kochsiek of Würzburg.

Simon led a strike of the scientific review commission of the Wissenschaftsrat in December of last year (see *Nature* 336, 506; 8 December 1988) to protest at the demotion of the council's general-secretary to the lowly status of a public servant. Pressure from the political heads of the *Länder* in late December pesuaded the federal government to restore the general-secretary position, currently unoccupied, to its original higher status.

In his final statement, Kochsiek pleaded with the *Länder* to increase their education budgets by twice as much as they had planned in order to alleviate overcrowding and a decline of quality at West German universities. S. D.

New NSF positions

Washington

RICHARD Nicholson, at present assistant director for mathematical and physical sciences at the National Science Foundation, was last week named as the new executive officer of the American Association for the Advancement of Science. He replaces Alvin Trivelpiece who left the association at the end of last year to become director of the Oak Ridge National Laboratory. Nicholson is expected to join the association by mid-April.

The foundation also announced last week that Mary Clutter would take over as assistant director for biological, behavioural and social sciences. She replaces David Kingsbury, who resigned last October amid allegations of conflict of interest over his dealings with a biotechnology company. J. P.

Accelerator all set

Washington

BACKERS of the Superconducting Super Collider, the proposed $4,400-million proton accelerator, now have everything they need except congressional approval for construction funds. Two weeks ago, Waxahachie, Texas, was confirmed by outgoing Secretary of Energy John Herrington as the site of the Superconducting Super Collider after a review of the full environmental impact statement, and last week the Department of Energy signed a nine-year contract with the Universities Research Association, a consortium of 66 universities, to manage and operate the facility. The association runs the Fermi National Accelerator Laboratory near Chicago, and was the only bidder. D. L.

Rifkin tries to block human gene transfer experiment

Bethesda

THE first experiment to insert foreign genes into humans, which received approval to proceed only two weeks ago, may be blocked by Jeremy Rifkin, an active opponent of genetic engineering.

On 30 January, Rifkin's Foundation on Economic Trends filed a suit in a US federal district court to stop W. French Anderson of the National Heart Lung and Blood Institute and R. Michael Blaese and Steven A. Rosenberg of the National Cancer Institute from carrying out the experiment. The plan is that tumour-infiltrating lymphocyte (TIL) cells marked with a gene for antibiotic resistance will be injected into ten cancer patients from whom the cells were isolated.

Rifkin has filed his suit because he says the approval process of the National Institutes of Health (NIH) did not fully review the experiment or allow for public debate in the final stage. But NIH officials point out that there has been ample opportunity for open discussion and the experimental protocol has undergone a complex and lengthy review process.

The road to approval for the gene transfer experiment has not been smooth. Anderson and his colleagues first proposed their experiment last June, and it was referred first to various institutional review committees and then to the NIH Recombinant DNA Advisory Committee (RAC) for approval. A RAC subcommittee on human gene therapy first concluded that more data were needed, but at the end of September, Anderson told the subcommittee that he did not have the data it was seeking, whereupon the committee deferred approval of the protocol.

But on 3 October, Anderson presented much of the same data to a full meeting of the RAC, which approved his experiment. NIH director James Wyngaarden ordered that the subcommittee be given a second chance to review the proposal in the light of the new data, and the application was afterwards resubmitted to the full RAC, which approved it by mail ballot.

Rifkin objects to the mail ballot. Andrew Kimbrell, policy director and attorney for Rifkin's foundation, says that after the 3 October meeting, significant changes were made to the experimental protocol which under federal law should have been debated in an open RAC meeting, adding that the RAC review process in this case has been "confused, backwards and illegal". Speaking at this week's RAC meeting, Wyngaarden countered that there were no substantive changes to the protocol approved at October's open RAC meeting, so a further public meeting was unnecessary. The gene therapy committee's final deliberations, held on 9 December, were public.

But Wyngaarden does agree that the Anderson case has pointed to a problem with RAC procedures. He announced last Monday that he would seek an amendment to the RAC charter requiring that protocols referred to a RAC subcommittee to be approved by that subcommittee before being put to the full RAC.

Anderson says he thinks Rifkin's lawsuit is "a good thing" because it will bring the issue out in the open for public discussion. But Rosenberg points out that an American dies from cancer every minute, and "any delay for any reason would be very unfortunate."

Rifkin also used the forum of the RA meeting to propose an amendment to NI guidelines that would establish a Huma Eugenics Advisory Committee to provid advice on the "ethical, philosophica social, economic and eugenic implicatio and impacts of human genetic therapy".

But RAC chairman Gerard McGarrit pointed out that the RAC does not hav the administrative authority to create suc a committee. Such amendments can onl be made by the Secretarty of the Depart ment of Health and Human Services wh approves RAC's charter. Nevertheless the RAC spent nearly two hours debatin the merits of the proposal, and althougl there was general support for the idea most felt existing review bodies coul cope with the issues that a eugenics com mittee would address. **David Swinbank**

Errors in US census rouse political protests

San Francisco

TIME is running out to resolve the political and legal challenges to the way the 1990 US census will be conducted. Yet practical issues such as how to count the minority population of large urban areas and whether to count illegal aliens will have a profound effect on the social fabric of the United States in the next decade.

The decennial census is used not only for allocation of congressional representatives among the 50 states, but also for the distribution of federal funds to states and cities for services such as hospitals, schools, highways and housing. But the census usually misses 10 per cent or more of the minority population in some urban areas, and the cities most hurt by the undercount have accused the Bureau of the Census of ignoring a proven statistical means of correcting the numbers.

Census bureau decisions are subject to strong political pressures, as a small change in population can mean the gain or loss of a congressional seat. Since 1970, the census has been taken largely by mailed questionnaires; enumerators are sent in person only to households that do not respond. The method runs into difficulties in poor urban neighbourhoods.

After the 1980 census, the census bureau faced 37 lawsuits by areas contending that their minority population had been undercounted, all of which were unsuccessful. Nevertheless, the bureau developed a method called the Post Enumeration Survey (PES), using an in-depth survey of 300,000 households drawn from representative city blocks, to adjust its first counts. A test of PES in Los Angeles demonstrated a 9 per cent undercount in black neighbourhoods.

Barbara Bailar, chief of the team that devised the method, acknowledges that PES will still miss people who actively avoid being counted, but says it will nevertheless bring the numbers closer to the truth. Despite endorsements from three scientific committees, the census bureau has decided not to use PES to adjust the 1990 census. The bureau claims its existing methods are sufficiently accurate, and that PES would be too expensive.

Bailar resigned in protest at the decision, complaining that the census uses other, less-sound adjustment methods costing more than the $31 million required for PES. Some claim that the decision is politically motivated by Republican fears of losing congressional seats, as the undercounted populations typically vote Democratic.

Meanwhile, a coalition of cities and minority advocacy groups has launched a lawsuit to compel the bureau to shift to PES. A bill with the same objective introduced in Congress was killed last September by the addition of an amendment requiring the exclusion of illegal aliens from the census. The census does not at present aim to determine citizenship, and the census bureau is being sued by a coalition of congressmen who oppose counting illegal aliens for apportionment of congressional seats.

The census bureau defends the inclusion of illegal aliens on the grounds that the constitution requires counting all residents. In any case, says Jeffrey Passel of the census bureau, excluding illegal immigrants would be difficult because it would require the determination of citizenship, requiring all non-citizens to be matched against Immigration and Naturalization Service records, known to be in disarray. **Marcia Barinaga**

Hungary breaks science links with Romania over Transylvania

London

A LETTER from Romanian scientists appealing to Hungarian colleagues not to break off scientific relations was recently read at a closed session of the Hungarian Academy of Sciences. This emerged at a meeting of the Hungarian government's cultural and foreign affairs committee last month. But the letter came too late. Talks between the two governments on cultural, educational and scientific cooperation seem to have broken down over the issue of Transylvania.

The dispute between the two countries dates back to the settlements after the First World War, when the Treaty of Trianon awarded Transylvania to Romania. But the Hungarian presence in Transylvania goes back more than a thousand years — Magyars were there before occupying what is now Hungary in 895 AD. In the sixteenth and seventeeth centuries, Transylvania was for a time the heartland of Hungarian resistance to the Ottoman Empire.

The Romanian policy of de-Magyarization of Transylvania began in 1918. Since the Second World War, despite the establishment of socialist regimes in both Hungary and Romania, Romanian policies in Transylvania have continued, but Hungarians have been reluctant to criticize the Romanians in public.

The Hungarian "Bolyai" University at Cluj-Napoca (formerly Kolozsvar) was closed down (the Rector committed suicide) and, during the 1970s and 1980s, reports of discrimination against Transylvanian Hungarians in education and professional employment became increasingly frequent and alarming.

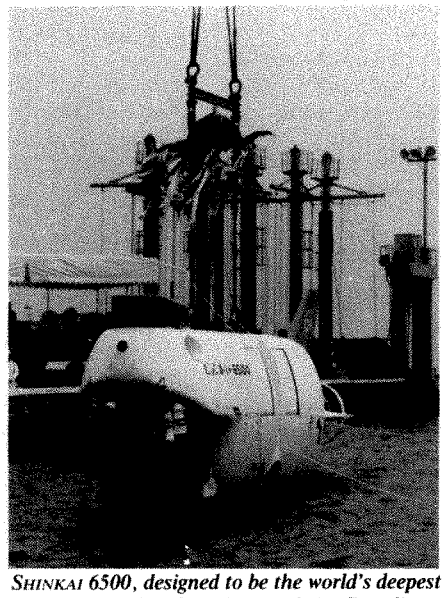
Since 1987, the Romanian government has been "consolidating" agricultural settlements, which means the destruction of the villages of Transylvania and the resettlement of the inhabitants in high-rise "agro-industrial settlements". Thousands of Transylvanian Hungarians have fled across the border, swamping the Hungarian social services. Private initiatives and action by the churches have, at best, offered only temporary relief. There are 2.3 million Hungarians in Transylvania but only 10.5 million in Hungary itself.

Diplomacy by the Hungarian government has come to naught. The Romanians inisist that the destruction of the villages is "rational" and "scientific" and point out that they have sent in "conservation" officers to photograph anything of interest before it is bulldozed.

Increasingly, the Hungarians are calling for an "international" solution. In an unprecedented move for a Socialist country, Hungary has invited a delegation from the UN High Commission for Refugees, which is due in Hungary next week. There have also been appeals by Hungarian "democratic" and "informal" movements in Hungary for the application of sanctions to Romania.

In Britain, there have been several calls for Madame Elena Ceaucescu to be stripped of the academic honours conferred on her by such bodies as the Royal Society of Chemistry. Although Ceaucescu is not, formally, responsible for the destruction of the villages, she is widely believed to be the real power behind the president. Although she is acclaimed as Romania's greatest scientist, others have questioned the quality and originality of her publications. But the Royal Society of Chemistry says that Ceaucescu was elected as a Fellow to the Royal Institute of Chemistry in the regular manner on the basis of her chemical career and publications, and that the charter of the Royal Society of Chemistry provides for the expulsion or suspension of a fellow only in the case of a criminal conviction, failure to observe the Society's bylaws or "conduct detrimental to the welfare of the society". **Vera Rich**

Sub's first outing



SHINKAI 6500, designed to be the world's deepest diving research submarine, took its first dip at Mitsubishi Heavy Industries shipyard in Kobe, Japan, last week. Its titanium alloy hull and pair of external manipulators will allow it to collect samples from the ocean bottom at depths of 6,500 metres, 500 metres deeper than vessels operated by other countries. After sea trials, the submarine will be delivered to the Japan Marine Science and Technology Center (JAMSTEC) in November. JAMSTEC will use the submarine to investigate the causes of undersea earthquakes, to assess deep ocean-bottom mineral resources and to study deep-sea life. Alun Anderson

New complaints about US waste in the Antarctic

Sydney

GREENPEACE in Australia has joined environmental groups in the United States in accusing the US government of leaving behind an environmental mess at research facilities in Antarctica. A scientific committee on Antarctic research, made up of scientists from Antarctic Treaty signatory countries, will meet next October to recommend new waste disposal practices for the Antarctic.

The Australian group is focusing on two bases in the Antarctic — Wilkes, a former US and Australian base, and McMurdo, the present US base. Wilkes was established by the United States in 1957 as a temporary base before McMurdo was completed. In 1964, the Australians took over the abandoned base, where they remained until 1969 when they moved to their present base, Casey.

According to a spokesperson for Greenpeace Australia, Lyn Goldsworthy, when Wilkes was abandoned "a lot of rubbish was just left behind". This included 200–300 petrol drums, many still full, old food sacks and derelict buildings.

In 1985, the US government gave Australia permission to clean up Wilkes. Rex Moncur, acting director of the Australian Antarctic Division, says that although the Wilkes buildings are technically US property, Australia accepts responsibility for cleaning up the site, and plans to start work at the end of this year.

Greenpeace in Australia claims that McMurdo is still generating a dangerous amount of waste. Goldsworthy says McMurdo's sewage system discharges directly into the sea, and that some parts of the ocean floor off McMurdo are heavily contaminated with polychlorinated biphenyls. She says Greenpeace has evidence that waste items such as batteries have been found at dump sites, contravening the code of conduct of waste disposal of the Antarctic Treaty.

Jack Talmadge, section head for polar coordination and information at the United States National Science Foundation, says the US government has always taken the waste problem seriously, and the recent reports have merely focused outside attention on the issue. Talmadge says the foundation's budget request for 1990 includes initial money for a four-year, $30-million safety and environmental health initiative that will clean up existing problems and establish new procedures and equipment to forestall new ones. "We're taking the high road in getting the job done", he says. **Tania Ewing**

Science literacy found wanting in United States and Britain

San Francisco

MORE than 90 per cent of adults in the United States and Britain are scientific illiterates, according to survey results released last month at the annual meeting of the American Association for the Advancement of Science in San Francisco. Its authors claim that it is the first such survey to be carried out in both countries, allowing comparisons to be made easily.

The dismal state of scientific literacy in the United States has recently prompted renewed attention to science education. The new surveys, developed in the United States, were designed to determine whether people have a minimal level of scientific literacy. To test for a basic understanding of scientific methods, respondents were asked "what does it mean to study something scientifically?".

Answers judged correct included testing of hypothesis and building of theory, the use of experiments, or systematic comparative study. As a check on this understanding, those who answered the question correctly were then asked to say whether astrology is "very scientific", "sort of scientific" or "not scientific at all". Only those who said astrology is not scientific at all were classified as having a general understanding of how science is conducted.

Simple questions such as "does the Earth go round the Sun, or does the Sun go round the Earth?" were used to judge understanding of scientific concepts. To assess understanding of the impact of science on society, respondents were asked questions such as "is it true radioactive milk can be made safe by boiling it?". (After the Chernobyl accident, a household in Cumbria in northern England was found to be boiling milk as a protective measure.)

Only six per cent of US adults and seven per cent of British were found to have at least minimal understanding of the process, concepts and impact of science. The US results were comparable to those of earlier surveys in 1979 and 1985.

Jon Miller, director of the Northern Illinois University Public Opinion Laboratory, who carried out the US surveys, blames the widespread scientific illiteracy on inadequate education, particularly at the high school level.

But some question the validity of the surveys, claiming that they tend to overestimate illiteracy because of misunderstandings of the questions. As an example, some members of the general public confuse astrology with astronomy.

There are interesting differences between Britain and the United States in questions relating to medicine. In a self-assessment, more US than British respondents (80 per cent against 50 per cent) said they are "very interested" in new medical discoveries. Similarly, about 70 per cent of US adults consider themselves "moderately" to "well informed" about such discoveries. But the high self-assessment does not translate into knowledge.

On several medical questions, US adults actually fared slightly worse than their UK counterparts. For example, 75 per cent of US respondents falsely believe that antibiotics are effective against viral infection, as against about 70 per cent of British. And although US adults were better at identifying risk factors for heart disease with more than 90 per cent correctly pinpointing smoking, lack of exercise, stress and eating too much animal fat, higher percentages of US adults (50–65 per cent) falsely believe that food additives and lack of vitamins, fresh fruit and fibre contribute to heart disease.

Miller says the dismal results in both countries are a "threat to democracy". He notes that science and technology are playing an increasing role in society but that the vast majority of people are not sufficiently literate in science to make informed decisions about major issues related to science.

Miller's solution is a radical improvement of the scope and quality of pre-university science education.

David Swinbanks

RVS move opposed

London

THE plan of the UK Natural Environment Research Council (NERC) to create a centre of excellence in oceanography at the University of Southampton will have to be redrawn to exclude the transfer of the Research Vessel Services (RVS) from Barry in South Wales, if staff at the base get their way.

The RVS provides the shipborne instrumentation, data acquisition and computer facilities necessary for marine research programmes of NERC and universities throughout the country. More than 60 of the 70 members of the RVS staff based at Barry are opposed to the move, according to one member of a committee formed to campaign against the relocation, who asked not to be named as staff have been asked not to speak to the press.

The committee has written to council members of NERC and to the secretaries of state for science and for Wales protesting against the plan, arguing that groups throughout the country rely on the services of RVS and that relocation at Southampton cannot be justified on the grounds of improved accessibility to the unit's services. They say that the move will cost £10 million, a sum that could be better spent on modernizing the equipment of the RVS. Creation of the centre at Southampton will cost £35 million over five years. The plan will go ahead if the government agrees to provide the money; an announcement is expected in the next few weeks.

Christine McGourty

Protection of endangered species threatened

Washington

PROGRAMMES to safeguard endangered species in the United States are themselves threatened by a lack of funds, says a report released at the end of January by the Government Accounting Office (GAO).

The Endangered Species Act of 1973 requires that the responsible agencies, either the Fish and Wildlife Service (FWS) of the Department of the Interior or the National Marine Fisheries Service (NMFS) of the Department of Commerce, should develop and put into action recovery programmes for species classified under the act. Although the GAO makes no attempt to assess the biological efficacy of these programmes, it finds that for 40 per cent of the classified species no recovery plan has been formulated, and that in the 18 cases it reviewed in detail only half of the steps in the plans have been initiated. The problem is compounded by failure of the FWS and NMFS to follow systematically the progress of its recovery plans.

Of the 482 species classified as threatened or endangered, fewer than one-sixth, according to the report, are increasing in numbers, and one-third are continuing to decline. The report also identifies a failure within FWS, which is responsible for 464 of the 482 classified species, to assign priorities to various species according to its own guidelines. This is partly due to pressure from Congress, which in 1987 forced FWS to spend 11 per cent of its recovery funds on only eight species, and partly due to a perceived need by FWS to provide a strong effort in some highly visible cases, such as the American bald eagle.

The GAO lays most of the blame on a simple lack of money, as well as organizational deficiencies in FWS and NMFS. But it also recognizes that recovery of some species may be unlikely, regardless of human efforts: the fragile desert habitat of the New Mexico ridgenose rattlesnake and the Texas beaches on which Kemp's Ridley sea turtle breeds are steadily shrinking, despite conservation efforts.

David Lindley

"Erosion of UK research on agriculture and food must end"

London

CUTS in government support for near-market research were severely criticised last week by the House of Lords Select Committee on Science and Technology. In a report on agriculture and food research in Britain, it said the government should reassess the list of projects defined as near-market so as not to include research which is in the public good, such as animal health and welfare, food safety and quality standards and protection of the environment.

The committee also recommends that the proposed timescale of three years for the transfer to industry of support for near-market research should be extended to five. Savings made in this exercise should be used to support basic and strategic research.

Although some of the rationalization in the Agricultural and Food Research Council (AFRC) has been to good effect, the cuts and the uncertainty about future



levels of support have caused instability and severe problems in the research community, says the committee. Industry should make a major contribution to the cost of research aimed at increasing industrial profitability and competitiveness, but the committee warns that if a sound research base is not maintained through public support, then commissions are likely to go overseas.

The research base in agriculture and food should not be reduced further, says the committee. A period of stability would lead to a reversal of the damaging trend towards more short-term appointments. Such appointments are appropriate when projects are of fixed length, but should not be used simply because future finances are uncertain. In the AFRC, the number of short-term appointments is already too high, it says.

The Ministry of Agriculture, Fisheries and Food comes under searing criticism from the committee. Scientific knowledge has had little impact on the ministry's policy, it says; because of the inadequate number of staff and an inappropriate structure, interaction with researchers is inadequate, as is the provision of proper guidance and advice.

The committee also criticizes the ministry's performance as a customer for research. Although it supports the principle that applied research should be supported on a customer contractor basis, its performance has been poor. Because of this, the committee recommends that funds transferred in 1975–76 to the ministry from the then Agriculture Research Council should be returned to the AFRC, or given to the proposed Natural Resources Research Council (NRRC). (In its interim report on agriculture and food, the committee recommended that a new council, the NRRC, be formed from a merger of the AFRC with the Natural Environmental Research Council.) The money — about £27 million — would be more productive as part of the AFRC or NRRC budget, says the committee.

The Biotechnology Directorate of the Science and Engineering Research Council is praised by the committee, but it says that the other research councils should be involved in the directorate's activities. There should be more joint initiatives, for which proposals should come from a new advisory group. It says that more research is needed on plant biochemistry and physiology, and it recommends more research on nutrition and forestry.

Christine McGourty

US plan for proton accelerator to produce tritium for warheads

Washington

AN old idea for producing tritium for nuclear warheads is gaining new favour in the United States. A report compiled by scientists and engineers at Los Alamos National Laboratory, Brookhaven National Laboratory and Westinghouse Hanford Company, which is soon to be submitted to the Department of Energy (DoE), advocates construction of a huge linear proton accelerator for the production of tritium; it may be powered by excess electricity from hydroelectric sources.

The closure last spring of the ageing Savannah River reactors, the sole US source of tritium for nuclear bombs, forced DoE to consider alternative production methods. Last summer, the department announced plans for two new production reactors, a heavy-water reactor at Savannah River and a modular high-temperature gas reactor at the Idaho National Engineering Laboratory (see *Nature* **334**, 558; 18 August 1988). The DoE budget request for 1990, compiled by the former Reagan administration, includes some money to start building the reactors. But a draft of the Los Alamos–Brookhaven–Westinghouse report claims that accelerator production of tritium would be cheaper and safer.

The principle of accelerator production is simple. A beam of high-energy protons is fired at a lead target to produce high-energy neutrons which in turn hit a lithium target which emits helium and tritium. The same approach is used in production reactors where the source of the neutrons is nuclear fission.

Wolfgan Panofsky, emeritus director of the Stanford Linear Accelerator Center, says the proposed accelerator is "well within the capabilities of present technology". In fact, he says, a prototype accelerator with a proton beam current comparable to that proposed (250 milliamps) was built at Livermore for the production of plutonium and tritium in 1949. Panofsky also agrees with the report's conclusion that the accelerator system will produce less radioactive waste than a production reactor.

But a key problem will be providing the gigawatt of electricity the accelerator will need. Building a new nuclear reactor to supply the accelerator would defeat the original purpose. But Pierre Grand of Brookhaven, who helped to compile the report, says that Bonneville Power Administration in Washington state, where advocates hope to site the accelerator, has tentatively said it can provide about 900 MW from excess hydroelectric generating capacity.

Another open question is cost. The report estimates that the 1.6-GeV facility will cost about $2,300 million with annual running expenses of $250 million — considerably cheaper than the estimated $6,800 million required for new production reactors. But Grand emphasizes that these figures are "very preliminary".

David Swinbanks

Genetics of schizophrenia

SIR—As is his wont, Steven Rose (*Nature* **336,** 512; 1988) belittles the efforts of molecular biologists to understand human problems. Rose's objection that "Sherrington *et al.* have not shown that [the locus on chromosome 5 to which their RFLP markers are linked] is sufficient [for a person to develop schizophrenia] — a prerequisite for useful genetic counselling — is misleading on two points. First, Sherrington *et al.* (*Nature* **336,** 164–167; 1988) did not claim that they had found a locus which inevitably leads to schizophrenia. They specifically quote a penetrance of 85 per cent even given a broad definition of schizophrenia-related diseases, stating that there are influences at work other than the genetic ones they have identified. Second, the locus is useful for genetic counselling whether or not it determines schizophrenia in the way that Rose would require of it. Genetic counselling has for many years relied on statistical arguments, often stemming only from mendelian genetics, with no linked markers at all. Many couples have felt that to be told that their children have a 25 per cent change of suffering from (say) cystic fibrosis is better than knowing nothing. Even with the advent of more statistically certain markers, such as the G8 probe which is linked to the locus causing Huntington's chorea, it is clearly recognized that possession of the locus is quite compatible with survival to 70 years of age with intact mental faculties. As always, genetic counselling is not only genetics — it is also counselling on the interpretation of those genetics.

This is exactly the reverse of the reductionism that Rose so vividly describes, but which is not actually present in your original articles: it is placing human interpretation on molecular results. That Rose invokes this argument only to knock it down as counter to a human science is at least consistent with itself, if not with the facts. That he then uses it to argue against attempts to develop more carefully targeted drugs to treat incapacitating mental disease is obtuse. Of course this course has not been successful in the past. It has not been available in the past. On this argument we would still be treating schizophrenia with the ducking chair and the stake.

WILLIAM BAINS

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Antarctic hero

SIR—I was appalled to see (*Nature* **336,** 417; 1988) that your Sydney, Australia, correspondent or your typesetter, or both, managed to spell the name of one of Australia's heroes as Sir Douglas Lawson rather than Sir Douglas Mawson. Any geologist who has ever handled a stromatolite knows his name (that includes me) and anyone with even the most meagre knowledge of Antarctic exploration is well aware of his exploits (that includes his great-grandniece, to whom I am married).

PAUL I. ABELL

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Reprints justified

SIR—I agree with Ivor Smith (*Nature* **336,** 708; 1988) that sending reprints is costly and time-consuming. In places such as Italy however, photocopiers abound, but university libraries do not subscribe to major journals, or journals are distributed only after months of delay. In such cases, it is vital for investigators to receive reprints (or even cheap photocopies) of papers within a few weeks of their listing in *Current Contents* or similar indexes.

MARCO RUGGIERO

Istituto di Patologia Generale,
University of Firenze,
Italy

SIR—I request reprints because it saves time. I subscribe to *Current Contents* and, on average, request three or four reprints per week, which takes about half an hour. If I were to go to the library, find the journals and copy the articles, it would probably take at least half a day. And often the journals that I need are not in the library because of budget cuts.

I feel that an investigator has the obligation not only to do research but also to disseminate it. Most administrators would probably be glad to provide the cost of photocopying and postage to have research at their institution known and cited.

M.B. KIRKHAM

1420 McCain Lane 244,
Manhattan, Kansas 66502, USA

Swiss academic strife

SIR—The picture drawn by John Maddox (*Nature* **336,** 333; 1988) of Swiss academic life is in my opinion a bit too rosy. I agree that there are a few positive aspects: faculty salaries, particularly those of senior professors, are high; retirement benefits are generous; SNFS research grants are easily obtainable. On the other hand, I do not share the view of ample opportunity for research. Minimum teaching loads for faculty at the University of Zurich, for example, are eight hours a week; an excessive amount of time is spent at *ad hoc* committee, departmental, faculty and senate meetings, occasionally lasting past midnight; and a similar amount of time is spent in exchanges with hordes of administrators at all kinds of levels of the education ministry of the canton.

As noted by Steven Dickman on the same page, student/faculty ratios are extremely biased in some fields. Even more crucial, it seems to me, is that as a by-product of a lax or non-existent admission policy, the range in quality of students is also extreme. This, in combination with the large number of students, has led to a decline in the quality of research and teaching, approaching mediocrity at best in some departments.

The bending of rules by cantonal officials to allow foreign academics to buy housing without having lived in Switzerland for five years is certainly a nice gesture. By contrast, once arrived in the land of Alps, foreign academics are often treated as second-class fellows by their Swiss colleagues. I believe there may be an analogy here to the treatment of women as second-class citizens in Swiss society. In 1984, Frau Elisabeth Kopp was elected by her parliamentary colleagues into the executive branch of the federal government, a first in Swiss history. How about the faculty of one or the other Swiss university electing one of their foreign colleagues to a deanship or even a chancellorship? Recently, Kopp was forced to resign from her cabinet post. I hope that is where the analogy would break down.

WALTER LEUTENEGGER

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Obfuscation

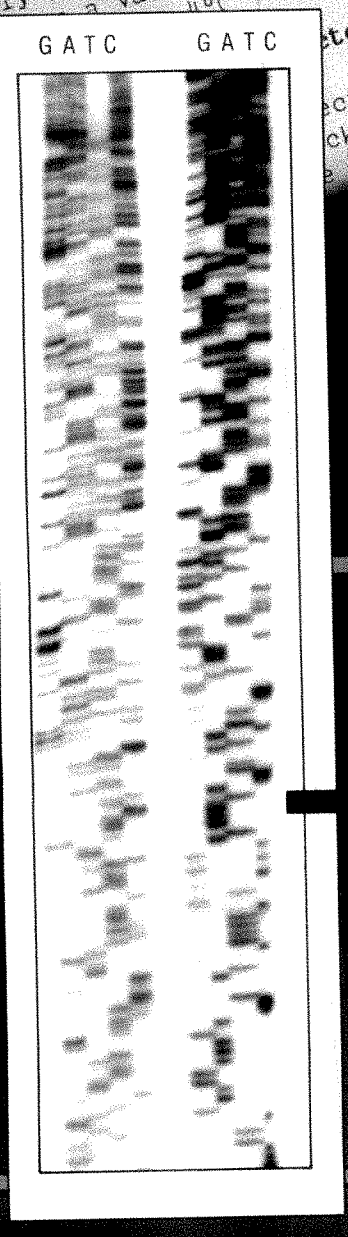
SIR—The recent letter by C. A. Smith *et al.* (*Nature* **337,** 181–84; 1989) seems to be an example of the apoptotic misuse of the Greek language. As before with other Greek words, I was unable to establish an unambiguous meaning of apoptosis. αποπτωσω is translated in the standard Greek–German dictionary as *verirren* (to go astray), so apoptotic might then be aberrant, and apoptosis could be translated by aberration. Using these meanings in the article, however, I was led astray: apoptosis as a process related to T lymphocytes, apoptotic as characteristic of these cells remained meaningless. The authors, however, seemed to like the word. But for a broad understanding of the article it is misleading. I am afraid that such use of cryptic Greek language by British scientists is a way of establishing barriers between them and others — deliberately, I wonder?

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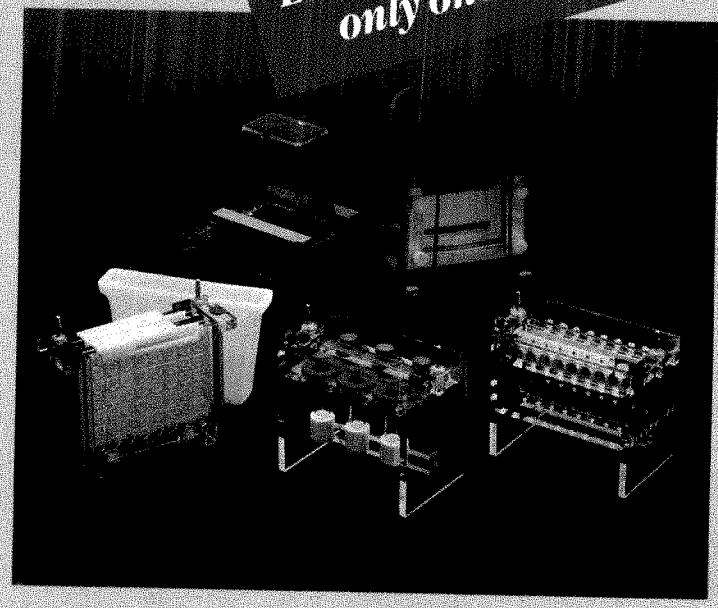


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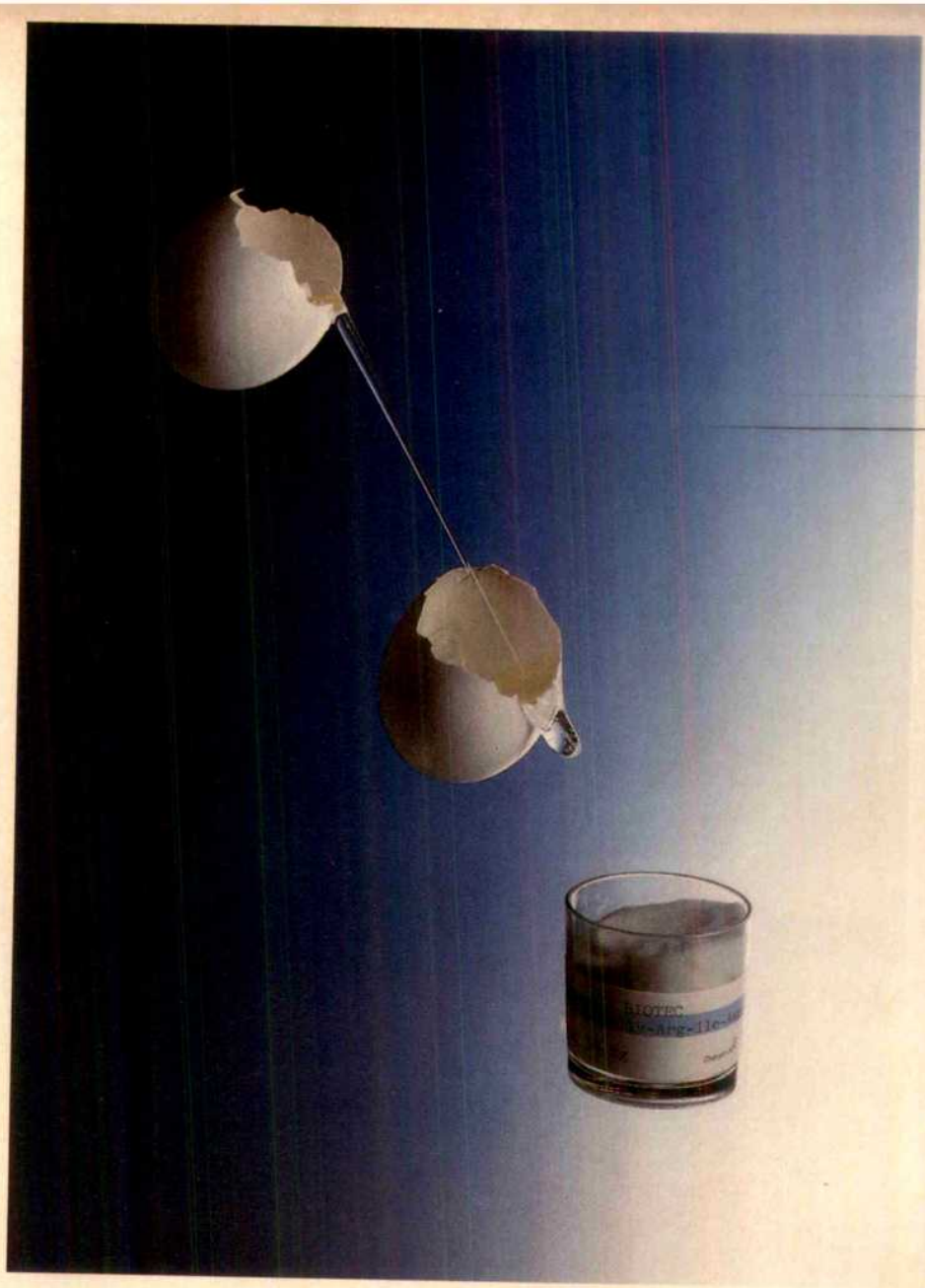
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More ways with neural networks

Ridding neural networks of some of their limitations seems to have suggested ways not just of accounting for the way that attentiveness can improve perception, but for human frailty as well.

PHYSICISTS, as eager as ever to believe that even biology is only applied physics, are naturally attracted to the notion that neural networks are a useful model for thinking processes capable of pattern recognition. One reason is that the formal description of networks is nicely mathematical. Another is that networks have already been used to solve other problems. An obvious, indeed an almost exact, analogy is that of the structures called spin-glasses, systems in which randomly distributed spins, magnetic or otherwise, interact with each other with a strength dependent on their random separations.

What could be more like the model of, say, the cortex, in which ON/OFF elements stand for neurons and in which the synaptic interconnections are described by coefficients whose magnitude determines the degree to which one neuron influences any other? Work on the spin-glasses was the first clearly to show that there is no unique equilibrium state of such a system but, rather, a large set of configurations, each of which represents a local minimum of the physical free energy.

In spin-glasses, these configurations are those in which the system may be trapped if the temperature is not great enough to allow random processes to discover and then to occupy another energy minimum with lower energy (which is the process of annealing). In neural networks, the same local minima correspond to the built-in patterns of the stimuli the network is capable of recognizing. Put crudely, a randomly chosen stimulus will first excite the network into a new state, which will then relax towards that built-in configuration to the stimulus most closely corresponds. In practice, this means that neural networks can recognize even very distorted versions of their built-in patterns.

But how well do the neural networks represent the real world of cognitive pattern recognition? While nobody expects utter precision from such inherently simple models, there are several respects in which they fail to correspond with everyday experience. All of us, for example, know that we can unconsciously, or even consciously, modulate the ease with which we recognize particular patterns by concentrating our attention on them: by what means do we adjust the coefficients representing synaptic strength in the manner this everyday observation implies? And what is to be said of the all-too-familiar situation that the cortex, when presented with some arbitrary stimulus, can only tell us that it cannot make sense of what it has perceived — that no one built-in pattern seems more appropriate than any other?

Two researchers seem now to have been able to adapt the standard neural network model to these needs, suggesting how it may be possible for a working neural network to adjust its own noise level and thus the ease with which particular patterns may be perceived. As a by-product, they suggest how the same modification may allow neural networks quickly to recognize which stimuli are novel.

Maciej Lewenstein and Andrzej Nowak, respectively a theoretical physicist from the Polish Academy of Sciences institute and a psychologist from the University of Warsaw, take the acknowledged defects of existing neural networks as the starting-point for their new construction (*Phys. Rev. Lett.* **62**, 225; 1989). Much of their argument is stimulated by the Monte Carlo computational techniques applied, for example, to the calculation of the stationary states of spin-glasses: by flipping randomly chosen spins in an initial configuration, it is possible to move systematically towards a nearby local minimum of the free energy. But the argument is, mercifully, not just another exercise in computational simulation.

First, Lewenstein and Nowak seek to distinguish between two kinds of built-in patterns or memories, some of which are more 'strongly' memorized than others. The operational difference is that between deep and shallow wells in a free energy surface, but the authors say it is the kind of categorization that might be found in hierarchically organized memories. How to organize such an arrangement?

The underlying assumption in the analogy between neural network models and the real cortex is that both structures must 'learn' which patterns to 'memorize', which requires that neural networks should discover what pattern of coupling strengths between different pairs of elements will embody a learned pattern in a stable fashion — and that neurons in the cortex should similarly grow appropriate synaptic connections.

What the authors find is that their networks will acquire a number of poorly 'remembered' patterns if equal weights are assigned to them in constructing the coupling constants between pairs of elements and if the well-remembered patterns are blessed with larger weights. Among other things, they find that the capacity of the network to recognize the poorly remembered states in an input stimulus is not impaired. But the unequal treatment does have the effect of reducing the number of the distinctive patterns the network may embody. In reality, there is no reason why the result (hierarchy) should not be advantageous in a real cortex.

The more striking innovation is that used for allowing the model neural network systematically to adjust its own precision, which is done by building into the equations rules that describe the way in which the noise level of the system is locally determined by the the lability of the network during its dynamic evolution.

The noise in the system must be likened to thermal noise, and determines the likelihood that it will spontaneously migrate from one local minimum to another that is more stable. But under the influence of an external pattern, all the spins will be shaken up (neurons will be switched off and on), whereafter the system will evolve to the remembered pattern most nearly determined by the stimulus.

The rules allowing the analogues of attentiveness to be built into recognition by a neural network are recurrence relations relating the noise at one point in the pattern-seeking process to that at an earlier stage by means of quantities representing the numbers of elements in the network changing their state in the interval.

Those working in the field will no doubt be eager to get their hands on the details. For the rest of us, the qualitative implications may suffice. Thus a neural network in the process of homing onto a remembered pattern will, anthropomorphically speaking, do so with increasing confidence. But a network presented with a stimulus that cannot easily be recognized for what it is will change the states of a great many elements in hunting for a solution, the noise will as a result increase and the network may, in effect, be saying 'don't know'. There is also the possibility that the state of a network faced with a badly chosen stimulus will as a consequence be impelled into a noisy state and then driven by random procesess towards the wrong attracting image state. In short, this adaptation of the standard neural network seems both to account for known psychological effects and for a good deal of human frailty — uncertainty and downright misjudgement.

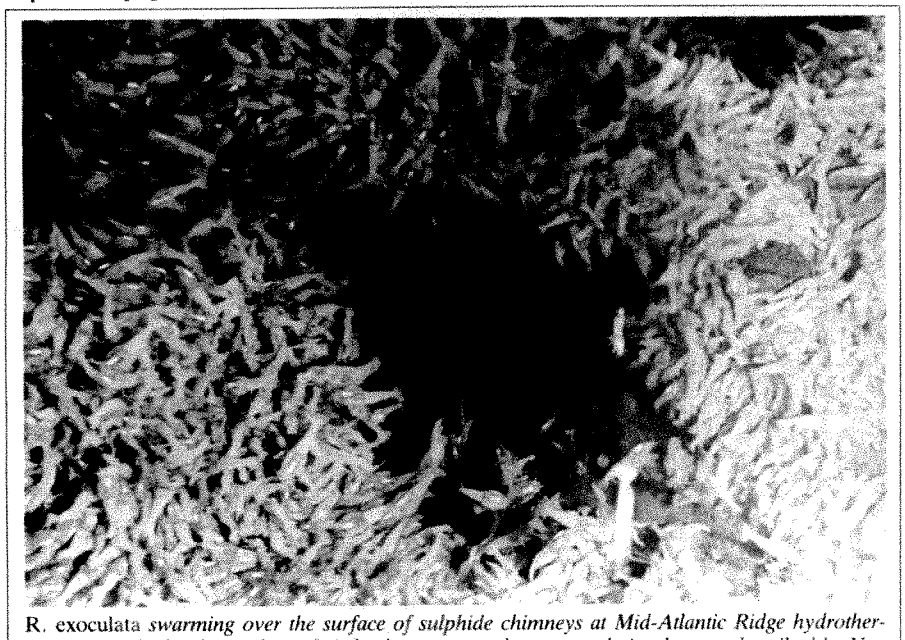
John Maddox

Animal behaviour

The sight of deep wet heat

Michael F. Land

DAYLIGHT disappears at a depth of about 800 m in the ocean. There are simply too few photons left for eyes to capture them in numbers large enough to provide statistically usable signals[1,2]. Below this depth most of the sparse fauna is eyeless. In the black depths of the Mid-Atlantic Ridge, 3.6 km deep, there are volcanic vents emitting very hot (350 °C) sulphurous water. Inhospitable as these sound, they are far from sterile. In particular they are inhabited by swarms of shrimp feeding on the sulphur-metabolizing microorganisms (see figure) that find the sulphide chimneys congenial. The strange thing about these shrimp is that they possess hugely developed structures that have much in common with eyes, as Van Dover *et al.* report on page 458 of this issue[3], even though the usual spherical eyes of ordinary shrimp have disappeared. What might be the function of these strange organs?



R. exoculata *swarming over the surface of sulphide chimneys at Mid-Atlantic Ridge hydrothermal vents. The bright patch on their backs corresponds to a novel visual organ described by Van Dover* et al. *on pages* 458–460 *of this issue. (Photograph by Andrew Campbell.)*

There is a considerable amount of bioluminescent activity in the deep sea, including near sulphide chimneys, so it is possible that these peculiar eyes might detect that. But, as Van Dover *et al.* point out[3], most animals that are known to use bioluminescence for communication have normal image-forming eyes. Indeed they must have, if they are to determine the directions of small sources of light. Rather than the detection of any conventional source of light, the authors suggest that these structures have a unique function: the detection of the sulphide vents themselves.

It is obviously a good thing for the shrimp to have a way of determining their proximity to a vent. Around the vent there is abundant food, but in it the shrimp would be instantly pressure-cooked. The question is whether or not the vents give off radiation that can be seen. I admit to a deep-seated reluctance to believe that columns of water can glow, related, I suspect, to my knowledge that water is for putting fires out. Nevertheless, the paper by Pelli and Chamberlain on page 460 of this issue[4] has educated me. These authors treat the vent simply as a 350 °C blackbody radiator, and work out how much energy there would be in the part of the spectrum to which the shrimp's visual pigment is sensitive. (Oddly, perhaps, the pigment is a normal blue-green sensitive rhodopsin, not especially sensitive in the red.) After heroic calculations, involving the estimates of pigment density made by Van Dover *et al.* [3], Pelli and Chamberlain conclude that the plumes would be detectable by an 'eye' of this kind, and at a distance of around 2.3 m for a 10-cm-wide vent. Pelli and Chamberlain also do the calculation for the human eye, and conclude that for us the luminance of the vents would be just below threshold — a result that squares with the initially damaging finding that dark-adapted observers in the Alvin submersible could not see them.

Why have the shrimp eyes abandoned their optics? Are such eyes actually better at certain detection tasks? For most vision, resolution is needed and optics are essential. But this may not be so for the detection of a single luminous source. The number of photons that can be captured and counted depends only on the area available to trap them, and this area can consist either of the pupil in an image-forming eye, or an area of naked retina as seems to be the case here. The naked-retina solution has the advantage of compactness; it is easy to fit a large detecting area into the carapace itself, whereas an eye of equivalent area would need to be large and three-dimensional. Provided only the crudest directionality was required, the eye's optics would in any case not be necessary.

If this shrimp, *Rimicaris exoculata*, were the only animal to have lost its optics while retaining the retina I would feel that the story presented in these two papers[3,4] was complete. However, there are actually many other naked-retina eyes among deep-sea animals[5,6]. These include other shrimp, isopods, amphipods, mysids and the famous fish *Ipnops murrayi* that turned up on the bottom of the sea at 3,500 m depth during the first deep-sea *Challenger* expedition of 1873. *Ipnops* has large, slightly concave retinas consisting mainly of rods, and covered simply by a cornea-like plate[7,8].

It is possible that all these animals live near hydrothermal vents, but this seems very unlikely. At these depths the only other use of the eyes would seem to be the detection of bioluminescence, but again, why abandon directionality when it would clearly be useful? The answer may be that these eyes (possibly including those of *Rimicaris*) are actually not quite as non-directional as they seem. A vertebrate rod, with a refractive index of 1.41, will trap light by total internal reflection over an angle of 36°, and a crustacean rhabdom of index 1.37 over 24°. In both cases, a curved array of these photoreceptors with no further optics could constitute an eye that would provide enough resolution for the rough direction of bioluminescent objects to be determined, as well as providing high sensitivity for their detection. It could be that we are dealing here not with naked retinas but with a new type of eye. □

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Nuclear physics

Engineering with quark matter

Charles Alcock

THE possibility that small drops of 'quark matter' might be stable was first put forward by Witten[1]. More detailed calculations by Farhi and Jaffe[2] showed that the hypothesis was certainly plausible; also these authors named stable quark matter 'strange matter' because of the important role played by strange quarks, a name which has stuck. The strange-matter hypothesis has several interesting consequences, principally in astrophysics (for a review see ref. 3). Two new papers, that of Brügger *et al.* on page 434 of this issue[4] and one by Madsen[5], address the question of how much strange matter there is in our part of the Universe. And Shaw *et al.* on page 436 of this issue[6] suggest a novel scheme for producing small lumps of strange matter, collecting and then growing the lumps; this is a proposal for engineering with quark matter.

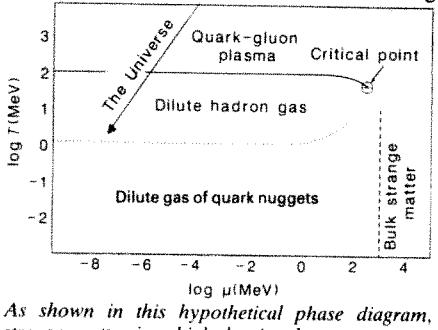
Quark matter is a hypothetical phase in which quarks are not confined in individual hadrons such as the proton (which consists of two 'up' quarks and one 'down') and the neutron (one up and two down quarks), but are free to move about within the phase. Strange matter is a quark-matter phase which contains three flavours of quarks — up, down and strange — and a few electrons. By hypothesis, strange matter is absolutely stable, which means that its mass (energy) per quark is lower than that of iron (which is the most stable ordinary nuclear matter) and cannot decay without violating energy conservation (see figure). This hypothesis is consistent with phenomenological models of the strong interaction, but good first-principle computations of the mass per baryon are not available.

Because strange matter is a bulk phase, it might be found in lumps of atomic mass A in the range 10^2–10^{57}, but with atomic number (charge) Z much less than $A/2$. These positively charged lumps are often known as quark nuggets. They behave chemically as ordinary atoms with the same Z and are, roughly speaking, nonstrous isotopes. A quark nugget may grow by absorbing neutrons; charged particles such as protons are not readily absorbed because of Coulomb repulsion.

For theorists, not being able to recognize the true ground state of the strong interactions (is it iron or strange matter?) is very disturbing. It makes sense, therefore, to search for quark nuggets or use experiments to settle the question. Madsen[5] proposes that observations of pulsars should help. Conventionally it is suggested that neutrons are the stable form of matter in the enormous gravitational field of these collapsed stars, but some argue that strange matter is. For his proposal, Madsen adduces the conclusion of Alpar[7] that the radio pulsars which exhibit glitches (abrupt, very small period reductions followed by a few months of relaxation) must be made of neutron matter, not stange matter. This conclusion rests on the success of neutron-star models for the glitch phenomenon and the absence of a model for glitches in the context of strange matter. Because strange matter absorbs neutrons, one seed of strange matter will convert an active neutron star into a 'strange' star. Madsen's analysis rests on the rate at which these seeds would be accreted from the interstellar medium.

Should they exist in the interstellar medium, quark nuggets will rain steadily onto stars, where friction slows them down. Thus a nugget can become bound to a star. If the star is a neutron star, the nugget will act as a seed which will grow until the entire star is converted to strange matter. Furthermore, if the star is the progenitor of a neutron star (a high-mass normal star which eventually explodes as a supernova, compressing its core to a neutron star), then any nuggets it captures will be present later when the neutron star is made. Madsen shows that this latter process is the most interesting and places limits on the number of quark nuggets in the interstellar medium. This is a model-dependent limit, of course, but it does show how to approach issues of fundamental physics using astrophysics.

A more direct method for seeking quark nuggets has been used by Brügger *et al.*[4], who have adapted Rutherford scattering, bombarding material samples with beams of ^{238}U and ^{208}Pb nuclei. Any back-scattering of the beams by angles greater than 90° indicates the presence of scattering centres (the nuggets) more massive than the projectile nuclei.



As shown in this hypothetical phase diagram, strange matter is a high-density, low-temperature phase (density increases with chemical potential μ). At high temperature, strange matter evaporates into ordinary hadrons (dotted line), much as a solid sublimates into vapour on heating; this transition is not a phase transition. At higher temperature, the hadron gas undergoes a phase transition (solid line) to a hot gas of quarks and 'gluons'. Cosmic or experimental production of strange matter occurs by producing the hot quark–gluon plasma with the hope that rapid cooling will trap portions of the matter in the cold, condensed phase. A detailed mechanism for accomplishing this has not been proposed. (Temperature T in megaelectron volts; 1 MeV ≈ 10^{10} K.) (From ref. 3.)

Brügger *et al.* applied this elegant technique to various natural samples (including a meteorite) and derive upper limits to the abundance of quark nuggets: 1 per 10^{10} for nuggets with $A \approx 10^3$ down to 1 per 10^{14} at $A \approx 10^7$. The technique is less readily applied for higher atomic number because the electrostatic potential of the nuggets is not a simple Coulomb form.

Shaw *et al.*[6] propose a much more direct approach to the study of strange matter. They propose that high-energy heavy-ion collisions of the kind studied in present CERN and Brookhaven National Laboratory experiments might make small quark nuggets. They further describe a scheme for isolating, slowing down and storing nuggets which are produced in the experiment. Any nugget which is captured may then be "grown" by the addition of low energy neutrons. This growth phase releases of order 20 megaelectron volts of energy per captured neutron — potentially a useful source of power, as this is nearly 10 times the energy yield per neutron in a conventional fission reactor. In this regard Shaw *et al.* are proposing a first attempt at engineering with quark matter.

There are uncertainities in this proposal. First, it is not clear that the CERN and Brookhaven experiments do indeed make a quark phase. Second, this phase will contain plenty of up and down quarks but strange quarks are produced only as $s\bar{s}$ pairs, and the strange quarks must separate from their anti-strange counterparts. Third, strange matter is a low-temperature phase and these experiments produce a very hot plasma (see figure). And fourth, the isolation scheme proposed looks for negatively charged nuggets, whereas strange matter is almost certainly positively charged in its most stable form.

Despite these reservations, the proposal does not seem to be an expensive addition to experiments that are already in progress. As the payoff in physics would be so large if strange matter were detected, the ideas should be further explored. And the new engineering ideas raise interesting long-term prospects for energy generation. □

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Peptide structure

Beyond transcriptional events

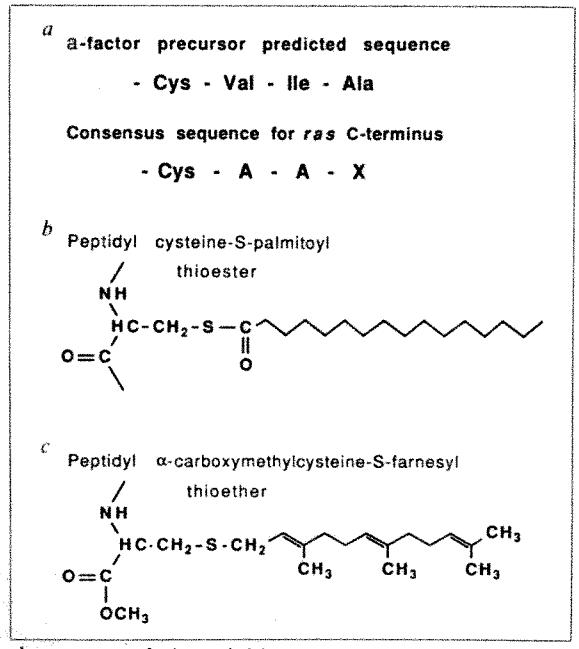
Anthony M. Treston and James L. Mulshine

PROTEIN chemistry, the demise of which was predicted in a News and Views article[1] 10 years ago, is still an essential technique for determining protein structure. Robert Anderegg and colleagues in the December 1988 issue[2] of the *Journal of Biological Chemistry*, for example, report the structure of a yeast mating pheromone using conventional protein-chemistry techniques. Their results demonstrate that, given the present lack of understanding of the mechanisms and control of post-translational processing, the prediction of processing events solely on the basis of nucleic-acid sequence data is speculative in the absence of supporting protein chemistry data.

The traditional approach for determining the structure of peptides and proteins has been through what could be called classical analytical methodology, including purification, derivation, hydrolysis and amino-acid characterization. Recently, however, these approaches have lost popularity because of the speed and precision of sequencing nucleic acids, enabling the prediction of the order of amino acids directly from DNA and RNA sequences. This 'molecular biology' approach to protein chemistry, although accurate, is confined to deriving the linear order of common amino acids in polypeptides.

Many peptides and proteins, however, contain amino acids which have been modified in various ways after translation from their messenger RNA, and these modifications cannot be derived directly from the primary structure of the gene. These post-translational modifications are essential for the biological function of the mature polypeptide, and both the types of modifications and the mechanisms by which they occur are apparently highly conserved across species ranging from yeast to man. The conservative nature of these modifications has allowed the determination of precursor protein consensus sequences which become the substrates for the post-translational processing reactions producing the mature molecule. The ability to predict polypeptide sequence from the primary nucleic-acid structure, coupled with knowledge of characteristic processing mechanisms, can in theory lead to the prediction of the mature processed form of polypeptides directly from a nucleic-acid sequence. But Anderegg *et al.*[2] demonstrate that a consensus sequence alone is not necessarily a reliable predictor of the structure of a mature peptide.

The nucleic-acid sequence encoding the mating hormone called a-factor from the yeast *Saccharomyces cerevisiae* has been known since 1985. The predicted precursor prohormone has at its carboxy terminus the sequence . . . Cys-Val-Ile-Ala, consistent with the consensus sequence for the acylation of the carboxy terminus of *ras* genes (*a* in the figure; sequences summarized in ref. 3). The identity of these sequences suggests that yeast a-factor should undergo the same post-translational processing as the yeast *ras*-related proteins. Furthermore, to exert their activities as regulators of cell proliferation, *ras* proteins must be inserted into the cellular membrane, and a similar mechanism has been postulated to be necessary for a-factor activity[3]. Membrane insertion occurs only for *ras* proteins that have been modified at the cysteine residue near the carboxy terminus by conversion into a thioester of palmitic acid[4]. These considerations all point towards identical post-translational modifications for *ras* proteins and a-factor, that is, conversion to a palmitate cysteine-thioester (*b* in the figure).



In fact, a yeast gene has been identified on the basis of being involved with the processing of both *ras* and a-factor. *RAM* (for *ras* and a-factor maturation function) was described[5] in 1986, and is essential for sexual activity of a-type haploid yeast and for the palmitate esterification and membrane localization of *ras* proteins. It was originally proposed that the *RAM* gene encoded an acyltransferase, with the *de facto* corollary that both a-factor and *ras* have the same cysteine-thioester modif cations. A gene called *DRP1* has no been shown[5] to be responsible for a pos translational processing step occurrin before fatty-acid alkylation of *ras*[5]. *DRP* turns out to be identical to *RAM*.

The data of Anderegg *et al.*[2] now unequ vocally show that the cysteine modifica tions of *ras* and a-factor are not identical or even similar. These authors purifie a-factor from *S.cerevisiae* and establishe by proton nuclear magnetic resonance an mass spectrometry that the biologica activity of a-factor results from a pair c closely related post-translationally modi fied peptides with a carboxy-termina α-carboxymethylcysteine-S-farnesyl ethe (*c* in the figure). Farnesol, a branched chain, polyunsaturated hydrocarbo alcohol, is best known as an intermediat in the biosynthesis of cholesterol an related molecules. The presence o a farnesyl moiety as a cysteine-S thioether *per se* is not unusual, as i is known to be a component of pep tide pheromones from other fungi Not only is the alkyl group of a-facto an unsaturated branched-chain alco hol, whereas the *ras* protein has a saturated linear fatty acid moiety but the chemical linkage is differen in each case; a thioether for a-facto and a thioester for *ras*. These finding are totally incompatible with the pre dicted identical modifications postu lated on the basis of identical carboxy terminal precursor sequences.

In retrospect, the incorrect predic tion of the nature of the a-facto modification and the identity of th protein encoded by *RAM* were caused by reliance on the 'molecula biology' approach to predicting pep tide structure. The *RAM/DRP1* gene described by Powers *et al.*[5] is obvi ously not an acyltransferase acting on both *ras* and a-factor. It seems more likely tha *RAM* encodes a carboxypeptidase o endopeptidase responsible for removal o the tailing A-A-X amino acids of both precursor molecules to provide a free cysteine for modification. Alternatively *RAM* could encode an enzyme respons ible for the methylesterification of th α-carboxy terminus of both peptides. The assignment of a function for *RAM* is an illustration of the need for direct deter mination of peptide structures to resolve the nature of proposed post-translational modifications. □

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Biochemistry

A protein with many functions?

R. B. Freedman

A QUIZ for the New Year. What is the connection between : (1) a gene product associated with visual transduction in the fruitfly *Drosophila*; (2) the major high-affinity binding protein in vertebrates for the immunosuppressive drug cyclosporin; and (3) an enzyme catalysing a rate-determining isomerization in protein folding *in vitro*? The answer, in two papers on pages 476 and 473 of this issue[1,2] and in a forthcoming paper[3], is that they seem to be the same protein. What this identity means will take some time to work out, because the protein, from whichever direction it is defined, is not yet securely identified with any specific physiological function. But the fact that it catalyses the *cis–trans* isomerization of peptide bonds involving the prolyl residue — the rate-determining step for the refolding of some proteins from the unfolded state — makes it tempting to look for crucial protein-folding processes in the immune response and in visual transduction.

Fischer *et al.*[1] and Takahashi *et al.*[2] in their papers in this issue have reached this point by asking essentially the same question. Both groups were attempting to characterize further the peptidyl-prolyl *cis–trans* isomerase (PPI) which the former group has identified and shown to be an abundant cytosolic protein[4]. PPI catalyses isomerization of the stereochemistry about the imide bond in the sequence -X-Pro-, both in small model peptides and in proteins, and in some proteins the result is catalysis of folding from the denatured to the native state.

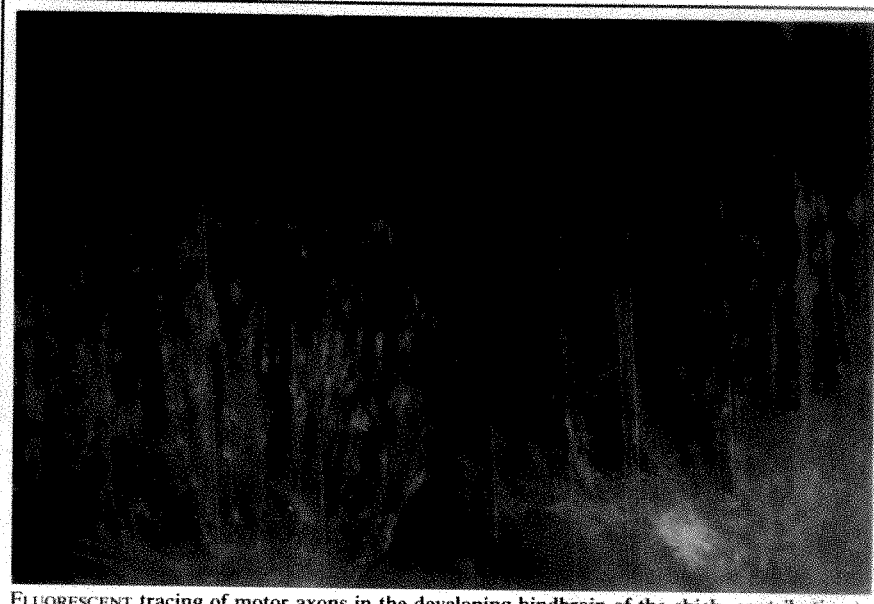
Fischer *et al.* have now identified[1] the 38 amino-terminal residues of porcine PPI using automated amino-acid sequencing. They find that this sequence is identical to the published amino-terminal sequences of cyclophilin from human and bovine sources. Cyclophilin was identified in 1984 as an abundant and widespread mammalian protein which binds with high affinity to the fungal product cyclosporin A, a cyclic peptide of 11 residues which is a powerful suppressor of the immune response. Because of the similarity of amino-terminal sequences, and of other properties such as molecular mass and amino-acid composition, Fischer *et al.* propose that PPI and cyclophilin are identical.

This proposal is confirmed by Takahashi *et al.*[2], who performed a complete protein-sequence analysis of PPI based on automated sequencing of peptides produced by trypsin, cyanogen bromide and V8 protease. Their results show that porcine PPI is identical in sequence to bovine cyclophilin and almost identical to human and rat cyclophilin, whose structures have been derived from the complementary DNA sequence.

Both groups[1,2] have pursued this surprising result to examine functional relationships between the enzymic activity of PPI and the binding activity of cyclophilin. They point out that previous results show both activities to be heat-sensitive, abolished by trypsin treatment and sensitive to modification of -SH groups. They now find that the presence of cyclosporin A inhibits the isomerase activity of PPI/cyclophilin and that the isomerase activity towards both peptide and protein substrates is completely inhibited by cyclosporin A at concentrations below 1 μM. Fischer *et al.* estimate an inhibition constant of below 10 nM, which is in the range reported for the dissociation constant of cyclophilin for cyclosporin A, as measured in studies of the direct binding. It is thus possible that PPI/cyclophilin is not just a high-affinity binding site for cyclosporin A, but is also the site of the drug's primary biological effect — the inhibition of the enzymic activity of PPI.

Where does visual transduction in *Drosophila* come in to all this? Charles Zuker and colleagues have been analysing the mechanism of visual transduction by characterizing mutations which affect this process in *Drosophila*. They constructed a library enriched for genes encoding proteins preferentially expressed in the adult visual system, and hybridized clones isolated from this library to polytene chromosomes to determine their positions on the cytogenic map. One of their clones (λ322) maps to the position 21D4-E2, within the position of a known visual transduction gene *ninaA* (located at position 21D3-E2 by conventional genetic mapping). The phenotype of the *ninaA* mutant is a drastically reduced level of the visual pigment rhodopsin, but there is no reduction in opsin expression. The mutation is therefore thought to affect post-translational modification of opsin or its degradation. By any criterion this is an interesting mutation, and it would be intriguing to know the nature of the *ninaA* gene product. Zuker and colleagues[5] now present the sequence of their clone λ322, and the inferred sequence of the *ninaA* gene product. This protein comprises 237 residues, and its central 180 residues are almost identical to cyclophilin/PPI; bovine cyclophilin/PPI has more than 40 per cent of the amino-acid residues, in common with the *Drosophila ninaA* protein, and conservative substitutions account for more than 30 per cent of the remaining residues. Given the taxonomic distance involved, the similarity is striking.

Taken together, the new reports identify an intriguing protein or protein family. In vertebrates, cyclophilin is clearly the main high-affinity binding site for cyclosporin A, and is thought to be its primary site of action; this action is not well-defined, but



FLUORESCENT tracing of motor axons in the developing hindbrain of the chick, contributing to evidence that growing nerves in this region respect segmental boundaries that may be analogous to those that define the body pattern of *Drosophila* during development (see the paper by S. Lumsden and R. Keynes on page 424 of this issue). The boundaries of the segments are preferentially stained with anti-laminin antibodies while the more central regions show preferential expression of the embryonic neural cell-adhesion molecule N-CAM. Strikingly, similar boundaries seem to be observed in the early embryonic mouse hindbrain by the expression of the zinc-finger gene *Krox*-20, which is expressed in alternating segments in the same region (see the paper by D. G. Wilkinson *et al.* on page 461 of this issue). □

it appears that cyclosporin A interrupts an early event in the antigenic activation of T-helper cells, leading to production of lymphokines. The new work shows that this protein is identical to the enzyme prolyl-peptidyl *cis–trans* isomerase, but the physiological function of this enzyme is not known. The presence of a closely related protein modifying the properties or stability of the visual pigment in insects raises a whole further set of questions. The power of gene and protein sequencing and the use of sequence databases have here connected three apparently disparate lines of work[1-3]. Now, more conventional studies on the properties of PPI, and on the roles of cyclophilin in the immune response and of the *ninaA* product in the visual system, will be needed to make sense of that connection in functional terms. □

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Optical computing

Polymers that make light work

Paul Calvert

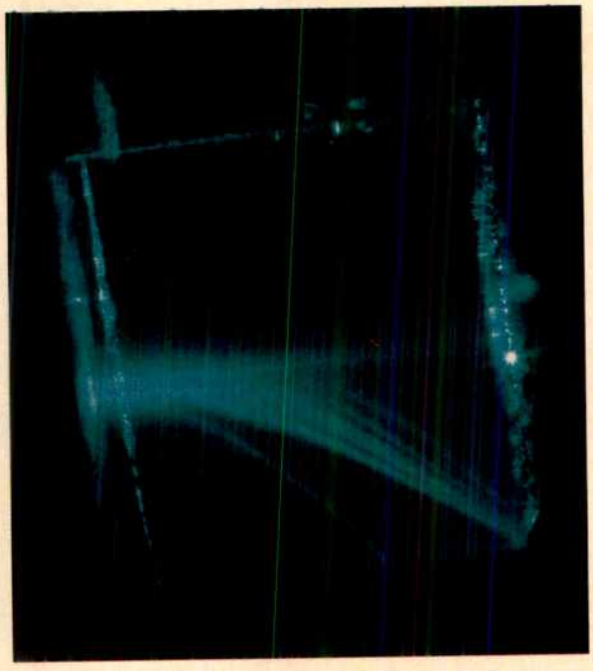
THE prospect of computing with light rather than electricity is attractive because light pulses travel faster in glasses than electrical pulses in metals, and light transmissions are less vulnerable to interference from outside, or from cross-talk between adjacent wires. At a recent symposium*, workers showed that they are close to developing polymers suitable for use in applications such as optical switches.

To make an optical computer, one needs ways of switching light beams on and off, or of diverting them between different paths. This requires a material whose refractive index changes either as a function of light intensity or as a result of an applied electrical field (see figure). The first effect would make it possible to build an all-optical computer, the second to use light to couple different parts of an electronic computer — an internal expressway. Conjugated organic molecules (with alternating double bonds) have large values of the important nonlinear optical coefficients and, although the simple compounds tend to give poor films, the equivalent polymers are readily processed into thin films for integrated optics.

The basic equation governing the nonlinearity is

$$P=\chi^{(1)}E+\chi^{(2)}E^2+\chi^{(3)}E^3 \ldots$$

where the polarization of the material P depends on the applied electrical field E to a series of powers. E can also be the oscillating electric field of light, in which case the first coefficient $\chi^{(1)}$ is related to the usual refractive index. The subsequent coefficients tend to be very small and so become important only with intense light, such as emitted by lasers. Although I will discuss them as simple coefficients, $\chi^{(1)}$–$\chi^{(3)}$ are tensors whose values are dependent on the directions of the input and output fields; $\chi^{(2)}$ is a third rank tensor (a three-dimensional array) and workers in the field are not yet ready to tackle the problem of defining the whole set (up to 27) of coefficients.



Bending light. The electric field of a laser beam induces changes in the refractive index of a barium titanate crystal which in turn bend the laser beam. Increasing the field intensity changes not the degree of bending, but rather the onset time for the effect, which is non-zero. (Courtesy of Malcolm Gower, Rutherford Appleton Laboratories.)

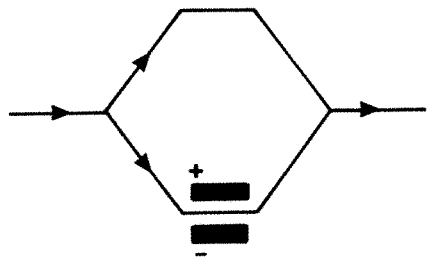
Consider the molecule as an organ pipe. It vibrates when excited by a beam of sound, even if the frequency is not the natural one of the pipe. A crystal is a room full of such pipes, regularly spaced. Sound travels more slowly through this than through air alone, as each pipe stores some vibrational energy and then gives it back. As the sound level is increased, the pipes become nonlinear and produce more overtones. The amount of second and third harmonic depend, through $\chi^{(2)}$ and $\chi^{(3)}$, on the properties of the pipes and their arrangement. Effects of $\chi^{(2)}$ will cancel out in a random or centrosymmetric arrangement of pipes.

The materials' requirements are: a big coefficient $\chi^{(2)}$ or $\chi^{(3)}$, no absorption of the light and little scattering so that as little light as possible is lost in each device, a fast response and easy processing into thin films. The material to beat is lithium niobate, which may turn out to be as ubiquitous in integrated optics as silicon is in electronics. At present it is damaged by the high intensities needed for all-optical switching, and although electro-optic switches can be made (see box), they are rather large because a long path between the electrodes is needed to produce sufficient effect on the light, and the response is rather slow. For a fast response, the effect must depend purely on the molecular electronic structure, anything involving atomic or molecular motion being too slow.

Crystalline, asymmetric organic materials exist with very high $\chi^{(2)}$ coefficients, for example 2-methyl-4-nitroaniline. The problem is that the crystalline films tend to scatter light at the grain boundaries, they are weak and they evaporate. Such compounds can be incorporated into amorphous polymers to give a relatively tough transparent film, but $\chi^{(2)}$ is zero unless an asymmetric structure can be induced. This can be done by applying a strong field across the film to align the active groups but the effect is still weak. Working electro-optic devices have been made from these polymers. At the recent meeting, J. Stamatoff, of Hoechst Celanese (which leads the field) described a polymer electro-optic switch that worked at volts but was 1.3 centimetres long. That is enormous by current computer standards.

Another approach is to forget $\chi^{(2)}$ and look for a large $\chi^{(3)}$, for which no molecular alignment is needed. Favoured materials are highly conjugated polymers. The extensive π-orbital systems make these the electronic equivalent of a rubber organ pipe and large $\chi^{(3)}$ values (around 10^{-} e.s.u.) have been measured for both polyacetylene[1] and the polydiacetylenes. The chief problem with polyacetylene is that it is black and so absorbs most of the

* *Materials Research Society*, Boston, 28 November–3 December 1988.



Pictured right is a Mach-Zehnder interferometer, one of several proposed switching devices[7]. Light is guided through a 1-micrometre channel between walls of slightly lower refractive index. The beam is split between two legs one of which has two electrodes above and below. Applying a voltage changes the effective refractive index in that channel so that the light slows until it is exactly out of phase with the other leg: when the beams are recombined, nothing emerges. This can convert electronic data to light pulses for transmission between parts of a computer that are too far apart for electronic conduction to occur rapidly. A similar logic device could have one leg intrinsically nonlinear such that the light in that leg slows as the intensity increases until it becomes out of phase with the other leg. This could then be part of an all-optical computer. The small channel size allows very high light intensities to be reached with relatively low total powers.

signal, but it is also intractable and unstable. As $\chi^{(3)}$ increases with the extent of conjugation to the fifth power, long conjugated chains should have huge values. Unfortunately these systems also absorb light.

The polydiacetylenes are coloured but transparent at long wavelengths and some can be cast as films from solution. They can also be prepared as Langmuir–Blodgett films (similar to soap films), but these tend to include light-scattering defects and the optically active groups are diluted by the soluble hydrocarbon tails needed at the processing stage.

Polybenzbisthiazole (PBT) also has a $\chi^{(3)}$ of around 10^{-10} e.s.u. (ref.3; Prasad, State University of New York, Buffalo). This is one of a group of rigid rod polymers developed from work at Wright Patterson Air Force Base on high-temperature polymers. Polyphenylenevinylene is another black, conjugated, conducting polymer and has $\chi^{(3)}=3\times10^{-10}$ e.s.u. when drawn into an oriented film.

The polysilanes, based on a silicon backbone rather than carbon, are an interesting possibility[4,5]. For polymethylphenylsilane $\chi^{(3)}=10^{-12}$ e.s.u. when measured with 1.06-micrometre light and decreases at longer wavelengths as the third harmonic is enhanced by resonance. Polysilanes are clear and can be easily made as films. They would be expected to have just a σ-bond system similar to polyethylene and no significant nonlinearity. The effect seems to arise from delocalization of electrons along the silicon-chain backbone which allows enough 'softness' to respond to the optical input. There is some evidence for this delocalization in the optical spectrum[6]. Also new data show that the response is very fast (taking less than 3 picoseconds) and is therefore due to electronic motion rather than molecular motion or thermal effects[5].

One expects the nonlinearity of an organ pipe to increase as the resonant frequency is approached and it starts to shake vigorously. In molecules resonance is usually associated with absorption and so is to be avoided. If the aim is to generate third-harmonic light, there should be no absorption at either the fundamental frequency or at the third harmonic. But to obtain a simple change of refractive index absorption at the third harmonic can enhance $\chi^{(3)}$ without detracting from the transmission of the fundamental. In general the measured $\chi^{(3)}$ rises as the fundamental wavelength is reduced until the third harmonic approaches an absorption band. In other words, absorption seems to be the price of a high $\chi^{(3)}$. Much attention was given at the meeting to molecular orbital calculations of $\chi^{(3)}$ which now can get within an order of magnitude of the right answer. We may be approaching a point where some useful prediction can be made of good structures.

Large inorganic crystals are already used for frequency doubling and tripling for laser spectroscopy, but these materials are difficult to adapt to small waveguides. Organic materials with extended conjugation offer large nonlinear coefficients and rapid switching when compared to inorganic materials. Polymers can be stable, easily formable and can (with difficulty) be made to high optical transparency. Whether these properties can all be combined in a single system remains to be seen. One view is that the stringent requirements of purity and stability in a computing system will prove to be too much for organics, as seems to be the case with conducting polymers. Another view is that opto-electronic transducers have been functioning effectively in chloroplasts and retinas for a long time and there should be no problem. □

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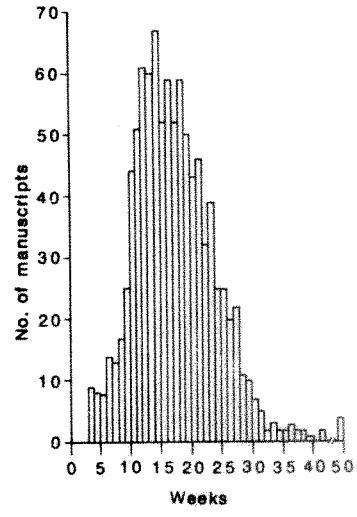
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Nature statistics

In 1988, about 6,000 unsolicited manuscripts were submitted to *Nature* for publication as Letters or Articles — almost exactly the same number as in 1987. About 35 per cent were sent to our Washington office rather than to London — there is no preference. About 30 per cent dealt with physical sciences rather than biological sciences. As usual, submissions peaked in the summer months to coincide with the period when potential referees (and *Nature* staff) are most likely to be away.

Of the submitted papers, just over 1,000 were published, 400 in the physical sciences and 600 in the biological sciences. If 1988 is no exception to the pattern of the past few years documented by the *Science Citation Index*, 30 of the 600 biological papers will be among the 100 life-science papers published in 1988 most cited by the end of 1989.

On the average, the delay between receipt and the appearance in print of the papers accepted for publication was just over 4 months. (Papers not accepted for publication are returned to their authors much more quickly.) As the figure shows, 30 or so papers, mostly on high-temperature



superconductivity, were published within 6 weeks of submission, but an equal number took more than 8 months to appear in print — sometimes because they were over-generously granted the original date of submission despite long delays during substantial revision. The median time between receipt and acceptance was 10 weeks, and from acceptance to publication was 7 weeks.

Naturally, we hope that publication will be even faster in 1989. One helpful development is the increasing use being made of telefax machines. For example, the proportion of referees' reports sent to our Washington office by that means increased from 8 per cent in June 1988 to 35 per cent in December. During 1989, we expect a further increase in the use of fax machines and some growth in the use of electronic mail to deliver reviews of manuscripts. □

Neuroscience

Cognitive control of movement

Roger Lemon

RECORDING from single cells in conscious animals has the potential to provide direct insights to the working brain. Investigation of cerebral events associated with movement using this approach has shown that cells in different areas of the cerebral cortex are involved with different aspects of movement performance[1]. Now, Georgopolous *et al.*, in the 13 January issue of *Science*[2], make the exciting claim that studying the activity in populations of cortical neurons can allow visualization of the cognitive processes preceding movement. These new results raise important issues about the central processing that occurs within the motor cortex before movements are executed.

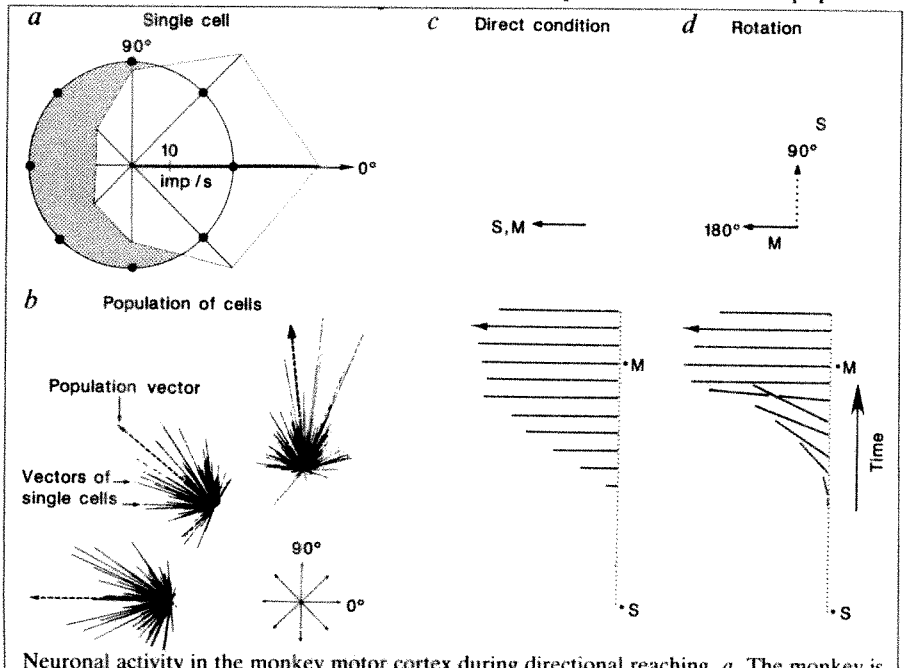
Georgopoulos *et al.* use mental rotation[3] as an experimental test of cognition in a rhesus monkey. In this procedure, the monkey is rewarded for making a movement at an angle to a target light whose position is changed from trial to trial. This process has been extensively investigated in humans[4,5], and it has been suggested that subjects solve the problem by mentally rotating an imagined movement vector from the direction of the stimulus to that of the required movement.

Georgopoulos and his collaborators have already shown[6] that the direction of movement is represented in the monkey motor cortex by the neuronal population vector (see figure legend). This concept is attractive because it concerns the contribution of a whole population of cells, rather than of individual neurons. In their new study[2], Georgopoulos *et al.* recorded the activity of single cells in the motor cortex during performance of the task and computed the behaviour of the population during the reaction time. They find that the information content of the population vector is greater than would be required to explain reaching performance in humans[7].

An important feature of the population vector is its predictive value. Mos neurons in the motor cortex are activ before the movement is performed, an the direction of the population vector is good indicator of the direction of th upcoming movement. Georgopoulos *al.*[2] trained the monkey to move eithe directly towards any one of eight targe lights (*c* in the figure) or at 90° in an anti clockwise direction to the light (*d* in th figure). When the monkey moves directl to the light, the population vector point towards the direction of the movemen from about 125 ms after the target wa illuminated; in the rotation condition, th vector first points in the direction of th stimulus light, and then begins an anti clockwise sweep until, at about 225 ms the vector points in the direction of th required movement.

Georgopoulos *et al.* claim that this rota tion of the vector is the neuronal equiva lent of the cognitive operation carried ou by human subjects performing the sam task. They point out that anticlockwis rotation of the vector (through 90°) i much quicker than in the opposite, clock wise direction (270°) and allows th monkey to solve the task quickly. Th speed of vector rotation in this overtrainec monkey was 732° per second, which i somewhat faster than the speed at whicl human subjects can perform the task[5] Remarkably, the reaction time was th same for both conditions (260 ms after th target light was switched on). Thus in th rotation condition, only 35 ms elapsec between attainment of the 'correct' direc tion by the population vector and th onset of the appropriate arm movement.

Hierarchical notions of movemen control imply that other, 'higher' brai structures, which project to the moto cortex, are involved in the cognitive operations needed to solve the menta rotation task. But the new results show that motor-cortex activity reflects these operations apparently throughout the preparatory period, and that the moto cortex does not receive only the fina command to move (for if it did, the vecto would not rotate, but it would already be pointing in the appropriate direction wher it appeared). It is perhaps surprising to find evidence for these operations in the motor cortex, which has long beer thought of as an executive structure in



Neuronal activity in the monkey motor cortex during directional reaching. *a*, The monkey is trained to move a lightweight handle from the centre of a table to one of eight target lights (dots) located at the edge of the working surface at 45° intervals. **Single cells** discharge for reaches in all eight directions, but are most active for one preferred direction (length of lines in *a* indicate mean firing rate during the reach). *b*, The broad 'directional tuning curves' of single cells make it unlikely that individual neurons code precise movement direction. But **populations of cells** could: *b* shows the vector contribution of the activity of a large number of single neurons; line length indicates discharge frequency. The **population vector** (dotted line) can be thought of as the sum total of discharges or 'votes' contributed by all the directionally tuned cells in the population; most votes will be cast by those neurons for which the required movement is in their preferred direction. The population vector points in the direction of the movement[6]. *c*, *d*, Temporal progression of the vector from the time the target light is switched on (S) to the onset of the reaching movement (M). The cells first become active about 125 ms after S, and movement begins at 260 ms. For the condition in which both stimulus and movement are in the same direction (*c*), the vector points in the required direction at all times. For the alternative condition, where the monkey must move in a direction of 90° to the stimulus (*d*), the vector first points in the direction of S, then rotates until, at about 225 ms, it is pointing at M (from refs 2,6).

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movement control, an idea enshrined in the use of the term 'upper motoneuron' in clinical neurology.

The functional linkage of the cells sampled by Georgopoulos *et al.* to the muscles producing the reaching movement is unknown. The weighting given to all cells in the population vector is equal[6], although it is known that some of these cells are probably interneurons and others are output neurons, projecting to the brainstem and spinal cord. It is also paradoxical that the time delay between discharge of even these output neurons and the onset of muscle activity can be more than 100 ms, yet the descending pathways from the motor cortex to shoulder muscles can be traversed in a tenth of that time[8]. Discharge of these cells can also be dissociated from movement, because the introduction of a waiting period into the experimental protocol allows the vector to be seen without movement[9]. □

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Cosmology

The elusive anthropic principle

Marek Abramowicz and George Ellis

THE anthropic principle is a modern attempt to deal with a question as old as mankind: why are we here? It is posed in the particular sense: why does the Universe seem to have special and very peculiar properties supporting our existence? Is this by pure chance, or is there an underlying reason, a purpose, perhaps a special design? Although the participants at a recent meeting* were hard pressed even to agree on what is the appropriate way to approach the question, there was one proposal that observations of the homogeneity of the Universe could be used as a concrete test of a particular variety of the anthropic principle.

The notion of an anthropic principle arises from the peculiar circumstances that must occur for life to evolve during the lifetime of a universe. For example, the natural constants in our Universe not only allow for the existence of stable atoms from which matter is formed, but also (together with proper initial conditions of the Big Bang) lead to the formation of galaxies and stars, apparently vital features of life. The task that those interested in the anthropic principle have set themselves is to reformulate, in a useful way, the questions of whether this a fortunate coincidence, inevitable or even just to be expected.

It was apparent from the discussions that even this raises considerable controversy. Some participants maintained that the whole issue is not a real scientific question; others that it is a key issue in cosmology. It was clear, particularly during informal discussions, that this controversy has a philosophical as well as a scientific nature: opinions are based not only on the speakers' scientific knowledge or extrapolations thereof, but also to a great extent on their *weltanschauung* (world view).

L. Gratton (Rome University) was one of the few who argued that the anthropic principle is meaningless. This was an attack on the strong version of the principle rather than the weak one (see below). F. Hoyle (University of Wales) argued that far from being meaningless, the principle supports the steady-state theory of the Universe; and G. Coyne (Vatican Observatory) argued that it supports (or at least is congruent with) the existence of God. Although these, and several other, philosophical interpretations were all analysed extensively, the main debate at the meeting took place on more physical grounds, between the supporters of the strong anthropic principle and those of its weak version.

The strong anthropic principle claims that the Universe must admit intelligent life, not by chance but of necessity. The weak principle stresses that we, as intelligent observers, may see (and do see) some apparently very special properties just because of the selection effect: intelligent life can only develop in particular places and at particular times. The issue, on this view, is to determine what restrictions this implies. Most participants came out against the strong principle and for the weak one. Interestingly, J.D. Barrow (University of Sussex), co-author with F.J. Tipler of the stimulating book *The Anthropic Cosmological Principle* (Oxford University Press, 1986), seems to have abandoned the strongest version expressed in that book, the final anthropic principle: not only must life come into being in the Universe, but it must then persist to the very end.

B. Carter (Paris Observatory), largely responsible for the renewed interest in the theme, was mainly concerned to argue the indisputable nature and power of the weak version of the principle. He points out that some appearance of a purposeful direction in biological evolution is entirely predictable (without invoking any mysterious design mechanism) provided one bears in mind that in addition to the darwinian effects of natural selection one should also take into account the anthropic effect of self-selection; our observations are biased by the fact we live in a Universe where intelligent life has indeed come into being. Our failure to observe the type of alternatives in which intelligent observers are proscribed, self-evidently cannot mean that there is no chance of such alternatives occurring. Their existence is tautologically unobservable.

H. Reeves (Saclay) developed implications of this theme, looking at conditions for the emergence of complex structures in the Universe (a necessary pre-requisite for the emergence of life). The emphasis in this approach is very much a physical one, relating to the formation of such complex structures according to the known laws of physics. Thus both Carter and Reeves played down the question of what would happen in other universes with other laws of physics (the kind of issue raised in discussing the strong version).

Perhaps the strongest defender of a version of the strong principle was D.W. Sciama (International School for Advanced Studies, Trieste) who argued that there are three possibilities: life can occur through pure chance; because of some purposive design (God); or because all possibilities occur in some ensemble of universes that includes those where life can occur and others where it cannot. Sciama's thesis is that what is not forbidden is compulsory; that is, all logically possible universes must occur, disjointly from each other. In a sense this incorporates into cosmology Dostoevsky's remark: "If there is no God, everything is allowed."

The most surprising part of Sciama's argument is a suggestion that, although the other universes are all causally disconnected from the one we live in (and between themselves), there is a possible observational test to support the hypothesis of their real existence. Essentially, his argument is that if all logical possibilities occur, then our Universe should be no more special than needed; another universe with only slightly different properties would also be sufficiently special to support intelligent life.

Thus some of the properties of our Universe should be random in the above sense, or at least not particularly special. Hence we expect to find, with accurate enough measurements, some assymetries in the Universe that do not accord, for example, with Penrose's assumption of isotropy ('conformal flatness') at the Big Bang or Hawking's proposal of a particular special set of boundary conditions on the wavefunction of the Universe which imply both initial isotropy and homogeneity. He would regard his thesis as satisfied when initial anisotropies (which

The Anthropic Principle, Venice, 18–19 November 1988.

in principle can be estimated from measurements of anisotropies in the cosmic background radiation) are found to be greater than predicted by these special geometries.

This is certainly a controversial viewpoint; apart from the questions it raises philosophically, it needs technical development to be made precise enough for verification. In fact, it eventually leads to the major unsolved problem of a probability measure on the initial conditions for the Universe, without which all this kind of speculation is on shaky ground. □

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Palaeoclimate

Finer grains of truth

Peter D. Moore

POLLEN analysis of organic peats and lake sediments is used extensively to reconstruct past changes in vegetation and climate. But such studies have concentrated mainly on changes detectable on a timescale of centuries or even millenia. Changes over timescales of a few decades or less are usually considered too fine to be resolved by the technique. But several recent projects show that this is not always so: Green and co-workers[1,2] for example, not only show that fine-resolution analysis is possible, but also relate it to environmental variables such as short-term climatic fluctuations and fire frequency.

Several problems stand in the way of fine-resolution pollen analysis. In many lakes, sediment is resuspended and redeposited during periods of turbulence[3]: it can even be 'focused' into certain parts of the basin[4]. These processes mix materials over a period of years and, although the general trends in the fossil record may be preserved, short-term changes are masked and smoothed. In peat deposits, the main problem is one of pollen migration within the loosely packed upper layers of the growing peat mass. Experiments in which pollen is added to columns of peat suggest that vertical transport with water flow limits the resolution potential of the analysis[5].

Despite these concerns, some studies both on lake sites and in peat deposits do permit fine-scale resolution. In deep, steep-sided lakes, resuspension may not be significant and sediments can even develop annual laminations, demonstrating the stability of sedimentation. In peats, several studies involving close sampling, even in poorly compacted layers, show a pollen stratification that argues against vertical pollen migration. Van Geel and Middeldorp[6] have now analysed the stratigraphy of freshly formed peat in Carbury Bog, Ireland, which shows strong peaks in pollen from Ericaceae, Gramineae and *Pinus* in the top 10 cm of unconsolidated peat, formed over the past 10 years. If there were significant downwash of pollen through the upper peat layers, these peaks would have been lost.

Green and colleagues have developed techniques of comparing such fine-scale pollen changes with fluctuations in environmental factors in peat[1] and in lake[2] sites. By looking at the sequential records of pollen, charcoal and (independently obtained) rainfall data as time series, they can cross-correlate the data sets and also allow for lag times between the causative factor and its response in the pollen record. They find that certain pollen taxa, *Eucalyptus*, grasses and Compositae (Tubuliflorae), show a strong positive response to rainfall, and that even small variations in rainfall are reflected in the pollen stratigraphy. These are also correlations with charcoal deposition. *Eucalyptus*, for example, responds positively with a 5–10-year lag phase, suggesting that trees damaged by fire had recovered rather than being destroyed.

But the relationship between pollen and charcoal is complicated by problems of soil erosion and consequent enhanced pollen input following a fire. And the origins of the charcoal itself must be considered: Clark[7] has examined charcoal transport in relation to particle size and considers that fine particles (such as those found on microscope slides for pollen preparations) are transported over long distances and do not necessarily reflect fires in the immediate catchment. So correlations between pollen and charcoal must take into account the dispersal characteristics of both pollen grains and carbon particles.

These recent studies indicate that the technique of pollen analysis, both of peats and certain types of lake sediments, does have potential in providing fine-resolution information concerning vegetation changes and hence the variation of other environmental factors in time. □

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Daedalus

Diamonds forever

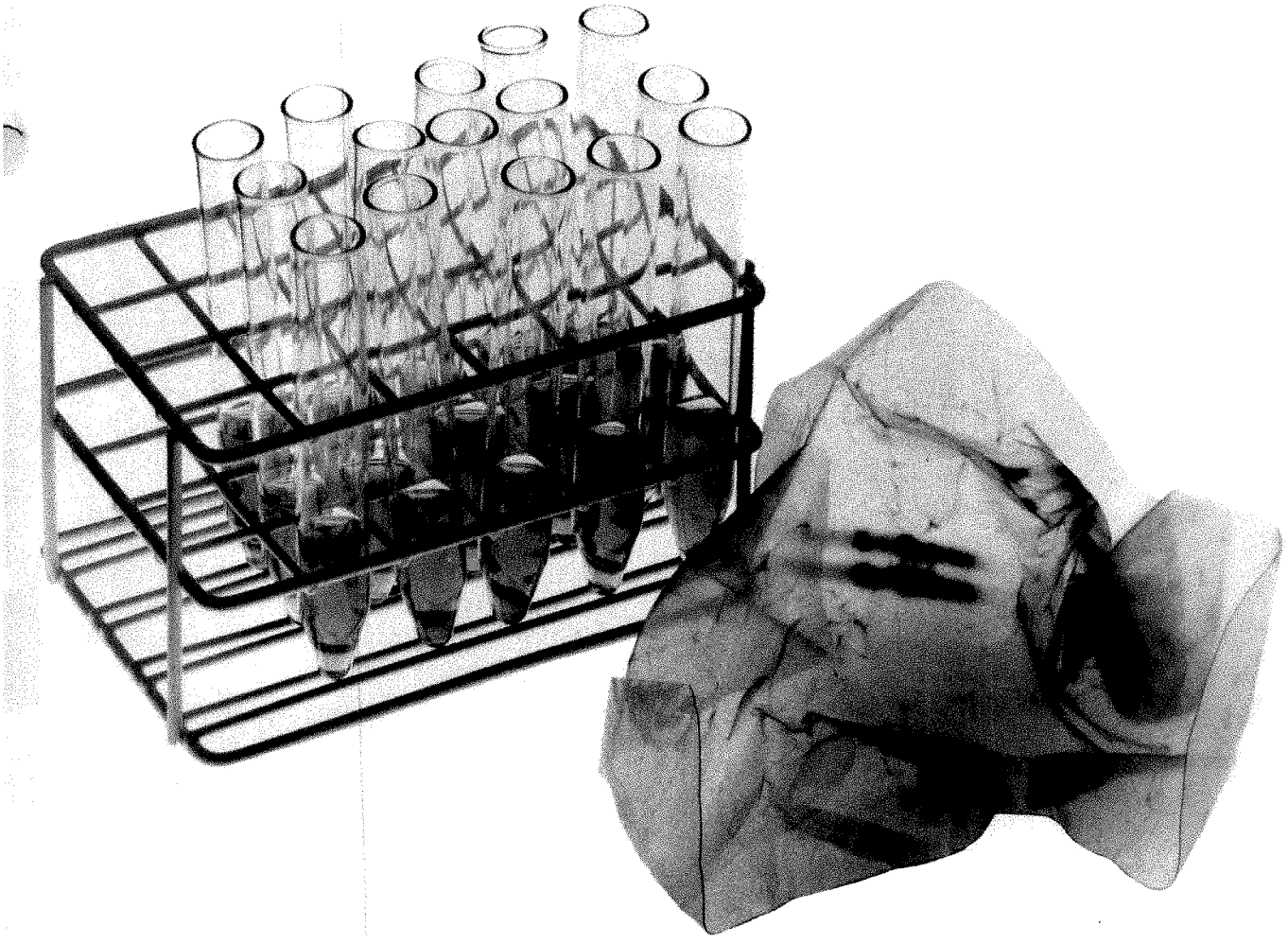
BRITTLE fracture is the ultimate engineering catastrophe. Microscopic cracks lurk in all solid surfaces; they gape slightly under load, and may even extend a little. Sooner or later the component may meet a stress great enough for a crack to run away, like a cloth tearing in two from a nick in one edge, and disaster strikes.

Now Daedalus has the answer. When a microcrack forms or extends, the local chemical bonds are pulled apart, leaving two pure free-radical surfaces. Such reactive faces must combine almost instantly with local air and water. But, says Daedalus, suppose the atmosphere around the crack were not air, but a polymerizable gas such as ethylene? Each radical-covered surface would be a splendid heterogeneous polymerization catalyst: the crack would be instantly bonded with polyethylene.

But polyethylene is not really an ideal 'glue'. Daedalus recalls that methane–hydrogen mixtures can deposit a tenaciously bonded diamond film on surfaces hot enough to decompose the methane to methyl radicals. A fresh crack surface dense with free radicals should do this even in the cold, by direct electron-transfer. A crack formed in a methane–hydrogen atmosphere should be instantly sealed with strong, stiff, inert, non-melting diamond: the finest imaginable engineering glue.

So Daedalus plans to 'crack-proof' industrial structures such as cranes, bridges and pylons, by slowly stressing them up to working load in an atmosphere of methane–hydrogen. As each microcrack in the structure opened up, it would be automatically sealed with diamond. When the structure finally reached its design load, each stressed part would be studded with tiny diamond inclusions: closing all its potentially deadly little cracks, and pre-stressing it perfectly to its service duty. Even structures like airframes, plagued by the growth of fatigue cracks in service might be 'immunized' against them by the sealing of the sites from which they begin.

And brittle materials like ceramics could at last be tamed. A ceramic turbine blade gradually loaded in hot methane–hydrogen would show its true strength at last. Deprived of the surface flaws from which to fail by brittle fracture, it could be loaded almost up to the point of plastic flow. Thus the splendid corrosion resistance and high temperature performance of ceramics will be made safely accessible to engineers. And the heat-stressed valves, blades, pistons and cams of modern high-power engines, so vulnerable even in metal, will be safe and durable at last. Even existing engines and turbines might be crack-proofed by pumping them up to speed with hot methane–hydrogen. David Jones



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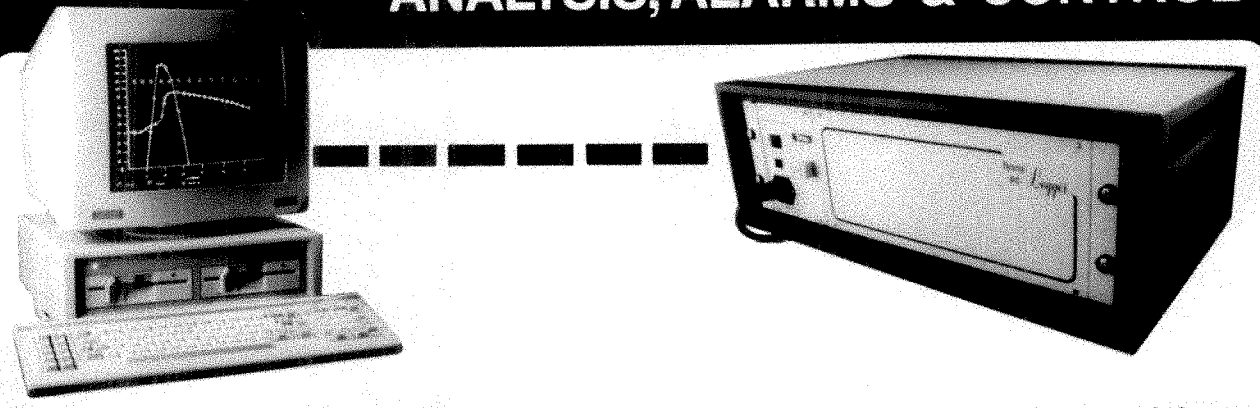
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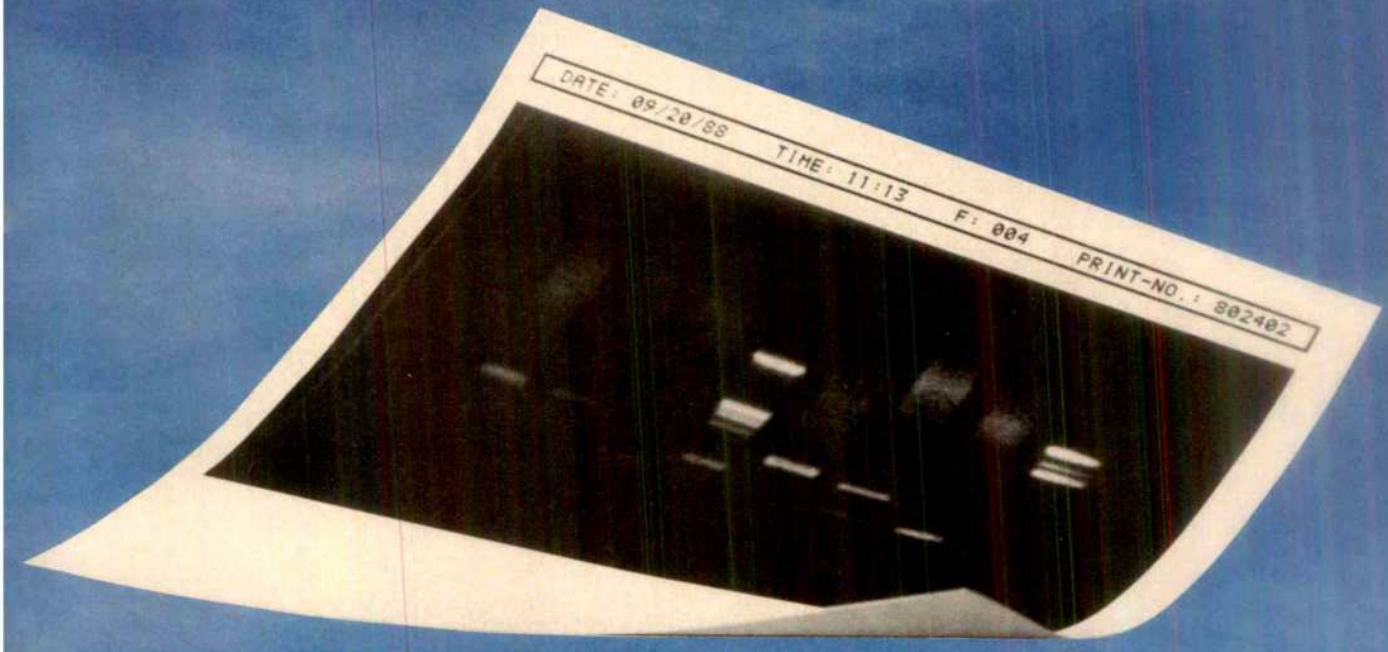
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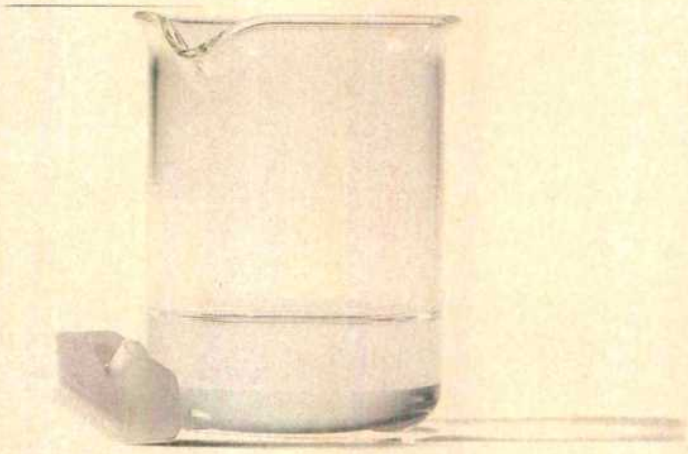


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Lethality of 'killer' bee stings

SIR—It has been predicted that Africanized honeybees (*Apis mellifera scutellata*) will displace European honeybees in warm climate areas of the United States after 1989 (ref. 1). In South and Central America, the highly developed colony defences of Africanized honeybees include a predilection for mass attack during which thousands of stings may be inflicted on one individual[2,3] causing life-threatening toxic reactions[4-9]. Although attacks of 300–500 stings have been survived without treatment[4], more than 500 stings are commonly fatal[8]. Strategies for minimizing the severity of reactions to Africanized bee sting attacks require information on the toxicity and quantity of the venom of Africanized honeybees. We report such data and conclude, by comparison with data from European bees, that the mortality associated with Africanized honeybee attacks is primarily the result only of the number of stings.

Worker (forager) honeybees (mainly *Apis mellifera ligustica*) were captured while returning to their hive in Tucson, Arizona. Attacking Africanized honeybees were captured from a colony in Guanacaste, Costa Rica[10] and identified by two independent morphometric determinations (H. Daly, University of California, Berkeley; S.W. Batra, Smithsonian Institution, Washington). Venom, free from contaminants, was obtained from the venom reservoirs by dissection as previously described[11], then lyophilized and stored at −20 °C.

Analysis of venom pooled from 1,000 bees of each type showed that European bees contained more venom (147 μg dry weight) than Africanized bees (94 μg), which were also smaller in size. The lethalities of the two venoms were calculated (as LD_{50})[12,13] from experiments in which Swiss mice and C3H/OuJ mice weighing 20–30 g were injected with venom in doses of 1,2,4 and 8 mg per kg, with eight animals per dose.

As shown in the table the lethality of the two venoms was not significantly different in Swiss mice challenged intravenously (i.v.) or intraperitoneally (i.p.), whereas Africanized bee venom was significantly less active than European bee venom in C3H/OuJ mice challenged intravenously.

Lethality of Africanized and European honeybee venoms in two strains of mice

	LD_{50} i.v. (mg per kg)	95% confidence interval	LD_{50} i.p. (mg per kg)	95% confidence interval
Swiss mice				
Africanized bee venom	2.8	2.0–4.1	2.8	2.0–4.1
European bee venom	2.8	2.0–4.1	3.8	2.2–6.4
C3H/OuJ mice				
Africanized bee venom	7.1*	5.7–8.8	ND	
European bee venom	4.6*	3.6–5.7	ND	

ND, not done.
* Significantly different, $P < 0.001$.

As the venoms from the two types of honeybee are of similar lethality, and as there is less venom in Africanized honeybees, the serious effects of multiple stings from Africanized honeybees seem mainly to be attributed to the multiplicity of stings. Perhaps use of the popular term 'killer bee' to describe the Africanized bee is inappropriate.

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Sexual advantage

SIR—Kelley *et al.*[1] report a 1.43-fold advantage for sexual over asexual reproduction in sweet vernal grass and thus seem to answer the question of "the ubiquity of sex." However, we find several problems in their methodology and analysis of the data.

First, in an attempt to simulate the natural dispersal pattern of the offspring, Kelley *et al.* planted sexual and asexual progeny conforming to the seed dispersal pattern around the parent. This is erroneous for two reasons. (1) Asexual progeny (tillers) are generally conjoint with the parents and do not disperse as widely as seeds. Planting asexual tillers in a pattern conforming to that of seed dispersal will grossly overestimate their fitness as it relieves them from competition for resources from the parent plant and also provides liberal space for the daughter tillers to sprout. (2) The natural pattern of distribution of the seedlings surviving to the age at which the authors transplanted the 'test' seedlings would be different from that of seed dispersal around the parent. Although the number of progeny is calculated, this is not used to arrive at the pattern of seedling distribution. Even if it had been, the failure to incorporate data on density-dependent germination and seedling survivorship at various distances from the parent would weaken the analysis. We believe that the planting pattern followed is very unnatural, and that the fitness estimates obtained are therefore incorrect.

Second, the relative advantages of sexual and asexual reproduction was compared using the number of inflorescences produced by the respective ramets. It denies logic to suggest that asexually reproducing organisms gain fitness through organs involved in sexual reproduction such as inflorescenes and spikelets. A valid method could be to compare the number of tillers produced by asexually reproducing ramets with that of equal-aged seedlings produced from the total spikelets of the sexually reproducing ramets. Clones of purely sexual and asexual types would be needed for such a comparison, but unfortunately they are not found in sweet vernal grass.

Third, the authors attempt to test "spatially varying environment as a selective force favouring sex" by comparing the relative fitness of the sexual over asexual reproduction for a 2-m distance from the parent. We do not think that the 2-m range is sufficient to represent a spatially varying environment; nor are we provided with any index of environmental variation within this 2-m radius around the parent plant.

Finally, the degrees of freedom for parents in Table 1 of ref. 1 should be 24 rather than 25.

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KELLEY *ET AL.* REPLY— Shaanker and Ganeshaiah seem to have misunderstood the purpose of our experiment. We intended to test the short-term advantage of sexual versus asexual reproduction, and not the advantage of seed versus vegetative propagation. Hence, we were interested in measuring the short-term selective force which would act against a mutant coverting sexual to asexual seed production (if and when it arose). Both

sexual and asexual progeny were thus planted in the locations to which seeds would naturally have been dispersed, because our hypothetical mutant presumably would not alter both the mode of reproduction and the mode of dispersal. The transplanted tillers were similar both in size and in age to seedlings which had germinated the previous fall in the field. We acknowledge that our simulation was a first-order approximation of the natural progeny dispersal pattern in the field, and that it did not incorporate data on density-dependent germination and seedling survivorship. As far as we are aware, few data on this point exist. Nevertheless, our previous experiments have shown remarkably little response of adult tillers and seedlings of *Anthoxanthum* to density variation in the field[2,3]. Further, the relative advantage of sexual progeny in *Anthoxanthum* is not strongly affected by changes in planting density[2]. We suspect, therefore, that our simulation of progeny dispersal is not particularly unnatural.

Shaankar and Ganeshaiah again seem to confuse asexual reproduction with vegetative reproduction when they argue that inflorescence number is an inappropriate measure of fitness. Hypothetically, asexual progeny could produce apomictic seeds, and hence gain fitness through inflorescences and spikelets. (Using the number of vegetative tillers as an estimate of fitness, our data still show a significant advantage for sexual progeny. The relative fitness of sex is 3.34 ± 0.71 when calculated using the number of vegetative tillers at the end of the second year as an estimate of fitness.)

Our experiment was not designed to test the spatially varying environment hypothesis, and difficulties in testing this hypothesis arise because distance from parent is confounded with progeny density. Nevertheless, the choice of 2 m as the maximal distance for planting was entirely appropriate in *Anthoxanthum*, as 99 per cent of all seeds is dispersed within 2.0 m. Hence, although experiments we have done do show a greater advantage for sexual progeny at greater distances (10 m) from the parent[4], such considerations are not relevant to explaining how a twofold advantage for sexual reproduction could arise, unless rare long-distance dispersal yields a considerable advantage for the sexual progeny, such as in a colonization event. The plant community within which the present experiments were conducted is relatively stable[5], and hence it is unlikely a significant short-term advantage for sex arises in this *Anthoxanthum* population from long-distance dispersal.

Shaankar and Ganeshaiah are correct to note that the degrees of freedom for parents, distance × parent, and parent × sex should be 24, not 25. However, the effect of this error was to deflate the *F*-values. The correct *F*-values are of higher significance, and our conclusions are unaffected. In summary, we believe our results are sound and provide clear evidence for a short-term (within a generation) advantage for sexually reproducing females.

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Thoroughbred breeding

SIR—Gaffney and Cunningham[1] and Hill[2] have addressed a biological dilemma with important consequences by illustrating that although TIMEFORM ratings for thoroughbred horses are increasing by about 1 per cent per year, the winning times for the English classic races have not changed appreciably since 1930. I propose that there is insufficient variation in speed realistically to expect significant reductions in winning times, and improvement in TIMEFORM ratings may be due to components of racing ability other than speed.

The genetic principle under investigation is that of a correlated response in winning time to selection based on TIMEFORM ratings. The expected correlated response per generation (CR) can be calculated from the formula[3] $CR_S = ih_S h_T r_G \sigma_S$, where S represents speed, T the TIMEFORM rating, i the intensity of selection, h the correlation between phenotype and genotype, σ the phenotypic standard deviation, and r_G the genetic correlation between speed and TIMEFORM rating.

One proposition to explain the contradiction is that the standard deviation for speed is very small. Data to compute this statistic directly are unavailable and so σ_S is estimated here from observations of trial races of 1,200 metres (six furlongs). A high proportion of horses trained for racing compete in such trials in which the distance between first and last seldom exceeds 70 metres. A typical winning time for 1,200-metre races is 72 seconds [4], and therefore a distance range of 70 metres represents a time range of about 4 seconds. Assuming that this range spans 95 per cent or 4 standard deviations of the population the standard deviation for time to run 1,200 metres is 1 second. From Gaffney and Cunningham $(i_m + i_t)/(L_m + L_t) = 0.132$ and $h_T = 0.6$. Substituting these values in Falconer's formula: $CR_S = 0.132 \times 0.6 \times 1 \times h_S r_G = 0.079\, h_S r_G$ seconds per year and taking 0.6 and 0.8 as arbitrary values for h_S and r_G respectively, $CR_S = 0.04$ seconds per year or 2 seconds per half century. This represents a response in speed of 2.7 per cent which is considerably lower than the 5 per cent predicted by Hill, but is still greater than the improvement indicated by Gaffney and Cunningham. But the predicted response will approach zero if the values of h_S and or r_G are lower than assumed above.

Why, then, have TIMEFORM ratings improved by 1 per cent per year whilst winning times have not changed significantly? A possible explanation is that breeders have difficulty in ranking animals across years. In this instance the problem may be compounded by TIMEFORM rating being subjectively assessed and influenced principally by a horse's ability to win races. Although such ratings may accurately rank animals within age group, their reliability as a measurement from which to estimate rates of genetic change should be addressed.

On the other hand, the improvement in TIMEFORM ratings may be a true reflection of genetic progress for ability of horses to win races. This 'ability to win' encompasses many traits such as strength, temperament, speed and desire to win. The fact that winning times are not changing may simply mean that speed has become a relatively unimportant component of 'ability to win' in thoroughbred horses during the past 50 years.

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CUNNINGHAM REPLIES—Eckhardt *et al.*[5] in their comment on our paper[1] suggest that the record times for the Derby in 1987 and 1988 provide evidence that racehorses are becoming faster. These two times do not invalidate our general point that winning times have been nearly static for more than 50 years. The main conclusion of our study[1] was in fact that genetic improvement in racing ability of the thoroughbred population as a whole has been very close to what selection theory predicts. We made the point that winning times of the best horses in the most prestigious races are not an accurate measure of genetic change in the population as a whole.

The speculations of Eckhardt *et al.* about the reasons for slow genetic improvement in thoroughbreds, although interesting, are not necessary, and are also debatable. Eckhardt *et al.* suggest that racing ability calls on such a wide range of structural and physiological factors, some of which are antagonistic to each other, so that improvement in net effect is more difficult than it is for economic traits in other domestic animals. A high-perform-

ing dairy cow, for example, secreting 3 kg of milk solids per day, makes comparably wide demands on her physiology.

Eckhardt *et al.* argue that genetic variation for running ability in horses may have been depleted by a long evolutionary history during which this was an important survival trait. Again, it could be argued that the evolutionary imperative was to outdistance predators, whereas the modern racing goal is to outdistance other horses. The first may have an intermediate optimum; the latter clearly is unbounded. Our estimate of the heritability of TIMEFORM rating, about 35 per cent, is similar to other estimates for different measures of racing ability, and all indicate substantial additive genetic variance.

How can the different question of static (or nearly static) winning times in classic races be resolved? One answer may be that tracks are now watered in dry years. Another is discussed above by Beatson, who suggests that selection on a subjective criterion like TIMEFORM rating produces a negligible correlated response in racing speed because of the very low variance in the latter trait. The low estimate of variability of racing time which he provides (CV=1.5 per cent) is supported by a similar value (CV=1.3 per cent) obtained by analysing many time records in greyhounds[6].

Another reason could be that winners in classic races are close to some physiological limit. Lactic-acid accumulation in blood rapidly reaches critical levels in horses undergoing continuous exertion[7]. This would impose limits in longer races but be less restrictive in shorter ones. There is a suggestion of a more pronounced plateau in performance in winning times in the St Leger (1¾ miles), than in the Oaks, Derby and Belmont (1½ miles), with even less evidence of a plateau in the Kentucky Derby (1¼ miles) and the Preakness (1 3/16 miles).

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HLA and insulin-dependent diabetes mellitus

SIR—The contribution of the polymorphic residue at position 57 of the HLA-DQβ chain to autoimmune diabetes (insulin-dependent diabetes mellitus or IDDM) has been discussed recently[1–5]. Genetic susceptibility to IDDM is positively correlated with a neutral residue (Ala, Val, Ser) and negatively correlated with an Asp at this position. Position 57 is also revealed as a critical residue in the analysis of *Pemphigus vulgaris*[6] but susceptibility to *P. vulgaris* seems to be a property of the allelic β-chain structure rather than just residue 57. Similarly, for IDDM susceptibility, there are several haplotypes with a neutral residue at 57 that do not confer high susceptibility (for example, DR7) and some with Asp 57 that seem to be disease-associated (for example, DR4 and DRw9 in the Japanese). Another challenge for the simple residue-57 model is how to account for the increased risk of DR3/4 individuals relative to DR3/3 or DR4/4 individuals as they are all non-Asp 57 homozygotes. These observations are most simply explained by assuming that other HLA class II sequences can also confer susceptibility to IDDM. The DRβI allele, for example, seems to contribute to the DR4 association with IDDM[7,8]. In general, it is specific combinations of class II alleles (haplotypes and genotypes) that are most highly associated with disease.

Notwithstanding these and other exceptions[9], the data implicating residue 57 as critical in disease susceptibility are striking: this intriguing correlation demands an explanation. One possibility is that the region around position 57 of the DQβ chain encoded by a susceptible allele (for example DQβ3.2) presents an autoantigen to the T cell or determines the expression of a particular T-cell receptor during thymic maturation[1,4]. The notion, however, that all allelic DQβ chains that contain Asp 57 (or non-Asp 57) bind a common islet cell autoantigenic epitope or influence the T-cell receptor repertoire in a similar way seems unlikely given the diversity of Asp 57 (or non-Asp 57) alleles (Table 1). An alternative interpretation is that the residue at position 57 may simply influence the overall conformation of the β-chain and not necessarily interact directly with a putative autoantigen. The functional significance of position 57 is suggested by the conservation of this charge polymorphism in all human class II β-chains (DQβ, DRβI, DRβIII, and DPβ). In fact, position 57 polymorphism at these other β-chain loci may also be relevant to disease susceptibility[4,5]. Moreover, the same polymorphic residues are found at position 57 in chimpanzee, gorilla, baboon, rhesus and cebus β-chain alleles (Table 2; Gyllensten *et al.* unpublished observations). The evolutionary maintenance over 10–20 million years of a balanced polymorphism between Asp 57 and Val, Ala or Ser at this position in all class II β-chains examined so far suggests that β-chains containing Asp 57 and those with Ala, Val, or Ser at position 57 may be structurally and functionally different. Perhaps, selection for the presence of both types maintains this polymorphism[10]. Whatever the functional significance of residue 57 polymorphism, the general correlation observed between disease susceptibility and the nature of residue 57 may ultimately reveal more about the structure of class II β-chains than about the specific response to a putative diabetes autoantigen.

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Table 1 HLA-DQβ sequences from residues 52 to 57

DQβ allele	Sequence	Group
DQβ3.2	—PLGPPA—	
DQβ2	—LLGLPA—	Ala, Val, Ser 57
DQβ1.1, 1.7	—PQGRPV—	
DQβ1.2	—PQGRPS—	
DQβ3.1, 3.3	—PLGPPD—	
DQβ4	—PLGRLD—	Asp 57
DQβ1.3, 1.4, 1.5, 1.6	—PQGRPD—	

Table 2 Residue at position 57 in class II β-chains

	DQβ	DRβI	DRβIII	DPβ
Human	Asp	Asp	Asp	Asp
	Val, Ala, Ser	Val, Ala, Ser	Val	Ala
Non-human Primates	Asp	Asp	Asp	—
	Ala, Val, Ser	Val, Ser	Val, Ser	

Primate species examined include chimpanzee, pygmy chimpanzee, gorilla, rhesus, baboon and cebus.

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Single-strand RNA

SIR—In their News and Views article, Lamb and Dreyfuss[1] state "All RNA viruses have to go through a ds RNA intermediate in their life cycle". If "ds RNA" designates double-helical RNA, this statement is incorrect, for the replication intermediate of RNA phage Qβ is a single-stranded minus strand, and not double-stranded RNA[2]. The same is thought to be true of many of the animal positive-strand RNA viruses, including picornaviruses, togaviruses and coronaviruses[3], although

the data are not clear-cut. There is no evidence that double-stranded RNA is an intermediate in the case of transcription and replication of negative-strand viruses (D. Kolakofsky, personal communication); rather, RNA is complexed to nucleocapsid protein, which probably prevents formation of double-stranded RNA.

Double-stranded RNA is nevertheless formed as a dead-end side product of Qβ RNA replication, presumably when there is a failure of the mechanism that separates or keeps apart plus and minus strands during synthesis[2]. In the case of eukaryotic minus-strand viruses it may be a similar rare event that gives rise to a double-stranded RNA that can be taken apart by the modifying/unwinding enzyme of Bass and Weintraub[4] and give rise to hypermutated RNA[5].

Were double-stranded RNA an obligatory intermediate of eukaryotic viral RNA replication, one would expect hypermutational events to be frequent, rather than very rare[5]. Maybe it is the avoidance of double-stranded RNA intermediates that enables RNA viruses to circumvent lethal hypermutation in eukaryotic cells.

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Human T-cell receptor expression

SIR—Janeway, in his recent News and Views article[1], stated that intestinal epithelial lymphocytes "in both chickens and man are predominantly CD8$^+$ T cells with γ/δ receptors". In our immunohistochemical studies of human intraepithelial lymphocytes, we also find that most intraepithelial lymphocytes express CD8, although approximately 20% of intraepithelial lymphocytes express neither CD4 nor CD8.

Using an antibody against the δ-chain of the T-cell receptor (δTCS1, T Cell Sciences Inc., Cambridge, Massachusetts) however, we have failed to demonstrate a predominance of δ-chain-positive lymphocytes in normal gut epithelium. We are unable to detect any δ-chain-positive cells in human fetal ileum at 20 weeks' gestation, when intraepithelial lymphocytes expressing CD3 are present[2]. We find that only 2 per cent of intraepithelial lymphocytes in normal adult jejunum, increasing to approximately 33 per cent in jejunum from children with newly diagnosed coeliac disease, express this form of the T-cell receptor. These findings suggest that accumulation of intraepithelial lymphocytes expressing the δ-chain of the T-cell receptor is antigen driven, but that in normal, immunologically stable intestine, the proportion expressing the γ/δ heterodimer is small.

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JANEWAY REPLIES—Spencer and Isaacson's statement that human intestinal epithelial lymphocytes may not be enriched in γ/δ T cells is further evidence of the need to correlate anatomy to structure and function. My statement regarding the presence of such cells in man[1] was based on results using the same antibody carried out by Bucy in Cooper's laboratory, and reported at the last FASEB conference in Las Vegas, Nevada.

I have since learned that another antibody of similar specificity also fails to reveal dominance of such T cells in gut epithelium in man (Groh *et al.*, personal communication). However, we now have evidence that intestinal epithelial lymphocytes of mice are using a V_γ:V_δ different from that found in any other tissue, including skin. Thus, one must be sure that the reagent used will react with all T cells bearing γ/δ receptors before such conclusions are validated. It is also possible that the results in the mouse reflect the early age at which such cells are collected. The γ/δ receptors in the gut and skin of the mouse are of a form found only very early in ontogeny in the thymus, and seem to rapidly seed these unique peripheral sites. The decline in such cells cited by Spencer and Isaacson may reflect this ontogenetic history.

However, the data of Spencer and Isaacson, and of Groh *et al.*, could reflect a major interspecies difference. If this last hypothesis is correct, would it be too far-fetched to speculate that the reason humans have spontaneous carcinomas, whereas mice seem to have sarcomas and lymphomas as their main tumour types, is that humans have a defect in the disposition of γ/δ T cells in their epithelia, thus allowing transformed epithelial cells to arise and proliferate sufficiently to invade and cause disease?

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Taxonomic stability

SIR—Crisp and Fogg[1] correctly point out that changes in names for taxonomic reasons, which arise from differing opinions on classifications or new data, are tiresome. Nevertheless, when changes arise from a more thorough understanding of evolutionary relationships they must be accepted as part of the development of science, as stressed by Newman[2].

The current International Union of Biological Sciences *Lists of Names in Current Use*[3] initiative aims to limit changes arising from the application of International Codes[4,5], rather than as a result of new scientific research. The most effective tool for restricting irresponsible taxonomic changes is peer pressure. Progress in that direction is starting to be made in the Ascomycotina, the largest class of fungi (6,100 generic names; 29,000 species). An interactive eclectic approach to the production of a generally accepted system for ranks from genus to order was initiated in 1982, based on a twice-yearly publication of this institute and the University of Umeå, *Systema Ascomycetum*, which reports changes in classification and reactions to them. Depending on world-wide peer reaction, changes are incorporated into an annually revised "Outline of the ascomycetes"[6], providing recommended dispositions of generic and higher ranks. Users have an annually updated listing to which they can refer. Parallel publications could clearly be initiated for other groups of organisms, were the necessary resources made available.

At the species level, the International Commission on the Taxonomy of Fungi (ICTF) produces a series "Name changes in fungi of microbiological, industrial and medical importance"[7]. The reasons for changes proposed in the naming of these fungi are reviewed by an international panel of 11 mycologists, and their views are embodied in this series. Again, this could be adopted on a wider front.

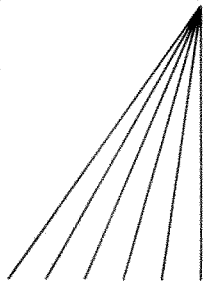
International peer reactions are the most likely prospect for restricting unwelcome changes of the type Crisp and Fogg abhore. In the longer term, improvement in the standards of taxonomic practice such as the ICTF "Code of Practice for Systematic Mycologists"[8,9], are required.

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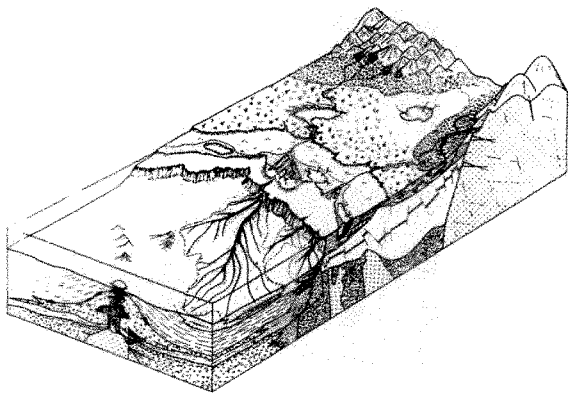
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Breaking resistance

ndrew Holmes-Siedle

uperconductors: Conquering Technology's New Frontier. By Randy Simon and ndrew Smith. *Plenum: 1988. Pp.326. $28.74, £16.*

o physicists and engineers, the lure of uperconductivity lay perhaps in its esemblance to magic. They had been ught that the consequence of passing lectrons through wires was heating. lthough true in the everyday world, ere was nevertheless a wonderland here the usual laws according to Ohm nd Ampère did not operate. It was a inter wonderland, a little chamber overed with icicles. The magic effect only ok place near absolute zero, requiring n expensive coolant, liquid helium. The irst attempt to harness the magic failed; a eneration of engineers who hoped to use uperconductivity in computers had their reams frustrated because of the price of ooling. Then, in 1986, materials scientists nade the first high-temperature super-onducting ceramic systems.

This breakthrough has caused an nusual flutter in the wider world. In part his is because some people believe that uperconductor magic has broken out of airyland and is about to transform our orld. There is thus a place for a book hich attempts to tell people how super-onductivity works and how it might, ndeed, change the world. Randy Simon nd Andrew Smith tackle this task in traightforward fashion. Their writing is nformal but makes no concessions when a difficult point is to be made. If readers have to stretch their imaginations a bit, the authors give them due warning and provide the tools of analogy to help them along. One reason for this confident style is that both authors are physicists who themselves work on high-temperature superconductors for an aerospace firm. They are in the forefront of their field, and have probably had to explain the same points to their managers a hundred times. At the same time, the writing is highly polished. This is no journalistic paste job.

Twelve chapters take the reader logically through the history, physics and engineering of superconductors, and describe both the old and new versions. The old, low-temperature (LT) superconductors are based on alloys of niobium, tin and lead, and are cooled by liquid helium or neon. The new, high-temperature (HT) varieties are based on cuprate ceramic compounds, and operate at temperatures near that of boiling nitrogen (high temperature in this context). These are dealt with in the final four chapters of the book, which discuss the excitement of the advances that have been made in the past three years. Chapter 13 gives a historical account of the initial discovery in 1986; and Chapter 14 describes the most popular research material, yttrium–barium–copper-oxide.

In the last two chapters the authors consider the future. Here, however, they have not done a real analysis of the uses and economics of the new materials, so their predictions are qualitative. But their views are logical — and agree with those of many reasonable people.

In the 1970s, the goal for the superconducting computer was to make a device, cooled by helium, that would fit inside a teacup and could reel off the names of every person in the world in one second. The efforts failed (the computer equipment looked like a kitchen stove), primarily because of the poor thermal properties of helium. Such computers had some highly specialized uses, including many military applications, but by the early 1980s research in the United States was faltering. The authors do not clearly explain why this was so, but they do spell out with enthusiasm the case that was being made for the continuance of LT research. This case was based on the advantages of the LT superconductor — speed of switching, absence of electrical noise and uniquely precise action in sensor devices.

The authors are confident that the discovery of HT effects will reverse the trend and revive research into LT superconductors. But they fail to mention a strong argument for continuing LT work, namely that the experience of workers in that technology might be harnessed to explore the possibilities of the HT effect. At the very least, LT research should continue while the basic physics and materials science of the new systems are studied.

We are by no means sure that the HT effect will do the things we want. Higher temperature is bound to erode one of the three advantages mentioned earlier, low-noise operation. Furthermore, the lattice structure of the new materials impairs electronic functions and may present insuperable barriers to widespread commercial success. We can only hope that the speed and precision of many classical superconducting effects are not affected too badly by this materials problem. Nevertheless, it will be surprising if the HT effects do not find some startling, novel uses.

Without trying to be prophetic, the authors set the scene for the growth of HT superconductors into a high-technology industry. Their well-balanced account of a complex technical challenge is likely to help laymen appreciate what is going on; many scientists who need a quick review of the subject will also want to read the book. If zero-resistance materials and new quantum-effect devices are going to come out of the winter wonderland and into the real world, then we all need to know about them. □

Andrew Holmes-Siedle is owner of REM Instruments, 64A Acre End Street, Eynsham, Oxford OX8 1PD, UK, and a consultant to the Fulmer Research Laboratories, Stoke Poges, near Slough, UK.



Face of the past — a Colossal Head, in total 2.84m high, fashioned from basalt and typical of the Olmec culture of Mexico (c. 1200–900 BC). The picture is reproduced from the new paperback of Michael D. Coe's Mexico, *3rd edn, to be published by Thames & Hudson on 13 February, £6.95.*

Question time for psychologists

Stuart Sutherland

Stevens' Handbook of Experimental Psychology, 2nd edn. Vol. 1 **Perception and Motivation;** Vol. 2 **Learning and Cognition.** Edited by Richard C. Atkinson, Richard J. Herrnstein, Gardner Lindzey and R. Duncan Luce. *Wiley: 1988. Vol. 1 pp. 905, $85, £77.50. Vol. 2 pp. 1,027. $95, £85. Two-volume set $180, £160.*

IN PSYCHOLOGY, old problems never die, they only fade away. On the other hand, new problems emerge at a remarkable rate. So it is instructive to compare the second edition of *Stevens' Handbook of Experimental Psychology* with the first, which was written nearly 30 years earlier.

The 'phi phenomenon', the illusion of movement occurring without anything being seen to move, received a great deal of attention in the first edition, but is not so much as mentioned in the second. As to the new and largely unanswered problems, here are two. First, when an ambiguous word (such as 'bank') is encountered, do people unconsciously access both meanings of it or are they directly guided by the context to the correct interpretation? Second, it has recently been discovered that people are very bad at probabilistic reasoning. For example, they fail to obey Bayes' law since they attach far too little weight to prior probabilities. Why this should be so remains unclear, although it may in part be due to a tendency to access the most salient material. For example, most people wrongly believe there are more words beginning with 'r' than words having 'r' as a second letter, because it is easier to recover from memory words starting with 'r' than words having 'r' in any other position.

Stereopsis

The failure to solve old problems and the constant emergence of new ones suggests that psychology makes little progress, but this is not entirely true. For example, in stereopsis the task of deciding which points on each eye are being stimulated by the same points in space (the correspondence problem) is an ancient one, but there are now plausible algorithms that can execute it. These receive only a cursory mention in the chapter on visual perception: unfortunately some psychologists still regard workers in artificial intelligence as rivals rather than allies.

Although the most secure advances may have occurred in vision and hearing, many discoveries have been made and a few theories put forward in other areas that are unlikely to be completely swept away. The notion that a concept may be based on a prototypical instance to which other instances bear a family resemblance goes back to Wittgenstein, but more recently it has been experimentally shown to apply to at least some concepts. As Eliot Hearst shows in an exceptionally clear and thorough chapter, even animal learning, currently a neglected field, has taken a dramatic step forward. It has been found that the formation of an association between two stimuli (for example, the conditioned and unconditioned stimuli) depends not merely on how frequently one is followed by the other, but on the second stimulus occurring more frequently after the first than in its absence. From a functional standpoint, this makes good sense. Animals, and people, need to learn about stimuli that predict an event better than other stimuli, not merely about random connections between the members of a pair of stimuli.

In psychology, the discovery of new phenomena has usually been determined by changes in the *Zeitgeist*, which in turn have often been influenced by the importation of new ways of thought from other disciplines. Elderly experimental psychologists, such as myself, have seen the subject successively transformed by cybernetics, information theory, Chomsky's generative grammars and artificial intelligence. All four movements suggest that it may be possible to describe and explain complex information-processing with rigour; hence the swing of experimental psychology away from behaviourism to a cognitive approach. The word 'cognitive' has been broadly interpreted because it includes not merely conscious mental processes but the unconscious ones that underlie them. Oddly enough it is the unconscious processes on which most progress has been made. Whereas there are plausible, but unfinished, theories about object recognition (a topic completely omitted from both editions), pitch perception, auditory localization and speech perception, theorizing on the higher realms of cognition remains vague. We have very little idea how memories are accessed and *a fortiori* how they are organized. And although it seems certain that reasoning proceeds by manipulating the semantic content of mental models rather than by the formal application of a logical calculus, we can hardly begin to specify what these mental models are like.

The influence of the information-processing sciences on psychology is new, but that of neurophysiology and related disciplines goes back many years. Today, hacking out pieces of animals' brains more or less at random has been largely supplanted by the study of neurological patients whose brains have suffered random damage through strokes, tumours or accidents. Such research is a secure and easy route to a PhD or an enlarged list of papers. But although it may provide information on where in the brain a given function is localized, it rarely yields any evidence that is relevant to the really important question of the nature of the mechanisms that execute it. Again, although Bartley Hoebel provides a detailed and scholarly account of recent work on neurotransmitters, the knowledge that they are heavily implicated in a variety of motivational systems, or even the more specific knowledge that the catecholamines play a role in the 'pleasure centres', does not appear to have advanced our knowledge of the mechanisms underlying reinforcement — let alone pleasure.

Experiments

The *Handbook* is not for beginners. It is written with the accuracy one might expect from the 40 or so distinguished contributors, but many of the chapters will be opaque to psychologists who do not themselves work in the field under review. There is little attempt to place a topic in perspective or to state clearly which problems (if any) have been solved and which remain unsolved, or even to decide what the important issues are. Instead, the reader is too often presented with a mass of experiments unadulterated by theory. Experiments are described that were intended to support theories long since shown to be fallacious — for example, those of A.M. Collins and M.R. Quillian that purported to show that the attributes of classes are held hierarchically, so that in memory yellow would be attached to canary, but flying would be attached to a higher node (bird). With several interesting exceptions, such as the chapter by S. Glucksberg and G.A. Miller on the psychology of language, most of the contributors seem to believe that if an experiment has been conducted it must be worth reporting.

Of course, experimental psychology has always lived up to its name and has tended to place more weight on experiments than on theory. The subject is, however, at present undergoing a fifth revolution, namely the development of simulated neural nets consisting of units randomly connected together, in which learning is represented by changes in the connectivity. Such nets have been found to be capable of performing surprisingly complex tasks and have been hailed as the theoretician's paradise. Unfortunately they are unlikely to introduce theoretical rigour into psychology if only because, for the most part, nobody understands how they work. □

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The view from Leningrad

A. Barrie Pittock

Climate Shocks: Natural and Anthropogenic. By K. Ya. Kondratyev. *Wiley: 1988. Pp.296. $54.95, £50.*

IN THE foreword to *Climate Shocks*, Will Kellogg describes the book as "an extraordinary *tour de force*" and "a scholarly achievement that is unmatched, to my knowledge". Unfortunately, I cannot agree. The book is certainly interesting to a relative insider, and it raises a number of points which deserve further attention. But, despite his eminence, Kondratyev puts forward a rather idiosyncratic view which will leave many readers bewildered rather than enlightened.

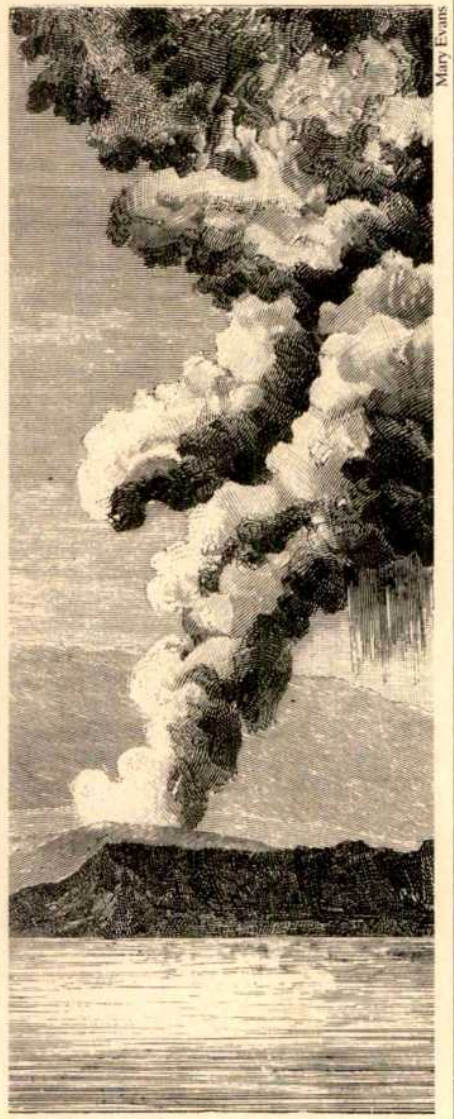
After a brief introduction to physical climatology, there follows an account of the greenhouse effect, and longer chapters on the climatic consequences of volcanic eruptions and on those that might result from a nuclear war. The latter contains a large section on the possible effects of the Tunguska meteor fall in 1908. A final chapter purports to present recent results and conclusions, but in fact largely summarizes the unrefereed papers that were presented at one particular conference held in 1985.

Throughout the book Kondratyev tends to give rather uncritical, detailed and technical summaries of selected papers, many of which are out of date, there being frequent confusion between the views contained in the original papers and those which the authors now hold. The result is a perplexing array of often contradictory statements, with too little attempt to mould the material into a rounded overview.

A central theme is the author's conviction that oxides of nitrogen, supposedly produced in large quantities by the atmospheric nuclear bomb tests of the 1950s and 1960s, caused a significant surface cooling in the Northern Hemisphere (an 'anti-greenhouse effect'), and that they would have caused a decrease in stratospheric ozone had it not been for a simultaneous injection of water vapour into the stratosphere. Kondratyev invokes the mechanism to reconcile the observed average surface temperature trends in the Northern Hemisphere in the 1930s through to the 1970s with the expected warming due to the greenhouse effect. But this requires him to add a contribution from industrial NO_x production before the bomb tests; as this input is tropospheric, the theory hardly stands up to close examination.

The NO_x theory is based on a controversial paper by Hampson (*Nature*, **250**, 189; 1974) and a time series of NO_2 concentrations derived by Kondratyev and coworkers by working back from the time series of ozone amounts. On p.177 he blames other people's failure to agree with his interpretation of the ozone record following the nuclear tests on rejection of the Soviet data obtained with the M–83 ozonometer; yet on p.192 he concedes that turbidity caused errors in the M–83 measurements.

Nitrogen oxides are also invoked to help 'explain' phenomena associated with the Tunguska meteorite event. Much space is devoted to explanations of the time series of atmospheric optical thickness over Mt Wilson, which shows peaks



Mary Evans

Perturbation for climate (and much else besides) — Krakatoa blows its top in 1883.

on 4 June 1908 and some 60 days later. The Tunguska event occurred on 30 June, so Kondratyev postulates another "pre-Tunguska stone meteorite" which supposedly disintegrated over Tibet on 4 June, the resulting cloud of particles taking 60 days to travel around the globe twice. Without any supporting evidence for the earlier event, this looks like special pleading. Indeed, the rather detailed estimates of the mass, velocity and other characteristics of the Tunguska meteorite, which Kondratyev says was a cometary nucleus composed largely of solid methane, seem to be plucked out of the air. Perhaps the primary literature is more convincing.

Kondratyev's account of the climatic consequences of nuclear war is largely built on selected early results, with an added emphasis on the possible effects of NO_x and other gaseous products. Like a number of other reviewers, he fails to identify clearly the critical role of absorption of solar radiation by carbon particles, as distinct from scattering. Use of the terms "optical depth" and "extinction", both of which include scattering as well as absorption, obscures the key point that most scattered light still reaches Earth's surface unless it is absorbed by the carbon particles. Thus on p.197 he quotes Crutzen and Birks as reporting solar radiation "extinction" by a factor of 2 to 150, when in fact they wrote that the "average sunlight penetration to the ground will be reduced by a factor of 2 to 150", which is quite different. This may be a fault in the translation, but it is a common misconception.

Other oddities may also be artefacts of translation — for example, a reference to 1815 as "a year without the sun" instead of "a year without a summer" (p.74).

With regard to the climatic consequences of nuclear war, Kondratyev, like many others, concentrates on the surface-temperature effects, giving too little emphasis to the perhaps more serious and robust results which show that rainfall would be dramatically reduced, especially in the tropics. He also fails to give proper attention to the importance of lofting of smoke due to its absorption of sunlight, and the resulting prolongation of the lifetime of the effects.

There is a natural and useful bias in the book towards the Soviet literature. But there is also a tendency to concentrate too much on results from Kondratyev's own group in Leningrad, and not enough effort is made at least to temper the view from Leningrad by comparing it with relevant non-Soviet studies. The treatment of the global carbon cycle is a case in point, with an extensive reference (p.12) to a controversial paper by Gorshov which claimed that "even after burning all existing fossil fuels, the CO_2 concentration in the atmosphere cannot increase by more than 35% to 40%". No contrary view is given.

In summary this is a confusing and somewhat partisan book, one which is perhaps more interesting for what it says implicitly about Soviet science than for what it contributes to an understanding of its subject matter. □

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World enough and time

David E.H. Jones

The World of Mathematics: A Small Library of the Literature of Mathematics from A'h-mosé the Scribe to Albert Einstein. Commentaries and notes by James R. Newman. *Tempus Books, 16011 NE 36th Way, Box 97017, Redmond, Washington 98073–9717: 1956/1988. Four volumes, pp.2,448 plus indexes. Distributed by Harper & Row, hbk $99.95, pbk $50.*

In 1940 James R. Newman, a lawyer and writer who had trained as a mathematician, began to assemble a popular historical anthology of mathematical writings. He reckoned it might take him two years. Fifteen years later he delivered the million-word manuscript to his publisher: 133 entries, each with a brief introduction and biography of its author. *The World of Mathematics* finally appeared, in four volumes, in 1956. It was immediately successful — a classic of mathematical popularization. Now Tempus Books has reissued it, still in four volumes but this time in paperback as well as hardback form. How does it read in 1989?

The coverage, eclectic from the first, is beginning in parts to date. The broad historical background of mathematics is still excellent; the sections dealing with mathematics in the physical and social worlds, and with the bases of statistics, stand up for the most part remarkably well. But many of the entries concerned with pure mathematics and its cultural relevance would certainly have been reconsidered by Newman today. The intellectual level remains demanding but not intimidating: appropriate for the enquiring non-mathematician, or the mathematician off-duty. About 20 per cent of the entries are drawn from the research literature, or at least the primary presentation of original work; 30 per cent are educational or polemical pieces; 40 per cent are popularizations of established mathematics; and 10 per cent are stories, or whimsical or discursive essays.

The charm of a good historical anthology is the variety of outlooks and challenges it presents. Here is Francis Galton in 1869 discussing hereditary genius, blandly contravening all the principles of social justice by proving that some people are inherently many times brighter than others. Here is Malthus's famous essay of 1798 on population, condemning most of mankind for most of time to unavoidable misery — and where's the flaw in his argument? Here is Alan Turing in 1950 on whether a machine might think, an amazingly modern essay which still reads cogently after nearly 40 years of massive developments in computing. Newman was clearly a connoisseur of good literary style. Each of his selections can be read purely for pleasure, even without bothering to follow its argument. Anybody who reads the scientific literature should enjoy the book for this alone; anyone who writes it will probably blush for shame.

The whole huge compilation is far too big to take in at a sitting. It is a book for extended browsing, or a rather eccentric work of reference. I like best the entries dealing with mathematics in the real world. Lanchester on the mathematics of warfare, showing how Nelson's brilliant strategy at Trafalgar closely approached a theoretical optimum; Moseley's account of the X-ray identification of atomic number, probably his last paper before his tragic and pointless death at Gallipoli; Bernard Shaw on gambling and insurance — a departure from his usual concerns, but characteristically spirited and surprisingly sensible; John von Neumann on the workings of the neuron, and the analogy between the brain and the digital computer.

This new edition has not been reprinted. A copy of the 1956 Simon and Schuster publication has been optically scanned and digitally reset (the stupefying intellectual triviality of the tasks that occupy most modern computers would have saddened Turing and von Neumann). The index has been slightly modified; I have spotted just two words of additional text. The publishers have thus missed the opportunity to remedy the most infuriating omission of the first edition: the lack of bibliographical references to the entries. Some can be placed from Newman's commentaries, but many are quite free-floating. It is quite impossible to stand in proper relationship to a piece of writing if you don't know in what context, or crucially *when*, it was written. The annoying detective work needed to pin down each entry from Newman's introduction to it, or from other sources, is almost the only unpleasing feature of this marvellous book. □

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Down to earth

Robert J. Devoy

Quaternary Geology for Scientists and Engineers. By John A. Catt. *Ellis Horwood: 1988. Pp.340. £39.50, $69.95.*

Quaternary geology (or, in a less partisan sense, Quaternary science) has long been the Cinderella of the earth sciences, attracting little attention as a discipline except from small national bands of enthusiasts. It is against this background that John Catt has produced a compact book of seven chapters that goes some way towards helping to redress the imbalance. The text presents information in a simple, practically orientated and narrative style, describing some of the subject's study themes, methodologies and techniques.

In some quarters, the book may not be received unreservedly. It is in places too much a catalogue of dry factual material. The treatment may also be viewed as overly selective and idiosyncratic, a situation which may have arisen from the author's aim of principally satisfying the needs of practical field scientists rather than those of specialists.

Against these criticisms, it should be remembered that non-specialists have to start somewhere. Despite the introductory approach the volume is not intended to be a definitive textbook, and the basic descriptive form will suit such non-specialist readers. Instead of a comprehensive overview, we have rather a helpful collection of working principles and definitions set within the framework of Quaternary data.

Quaternary science is an interdisciplinary and applied area of research. Its historical origin in nineteenth-century geology, coupled with the subsequent growth of specialisms, has too often led to a view of the subject as preoccupied with stratigraphy, gravels and an arid ordering of glacial–interglacial stages. It is refreshing, therefore, to see included here accounts of relevant specialisms which are clearly rooted in the aims and concerns of Quaternary studies. Geomorphology, soil science and dating techniques are all given chapter status alongside the coverage of basic stratigraphic approaches. A further innovation is the inclusion of chapters on mapping techniques and on the economic and applied importance of the Quaternary studies approach.

Regrettably some relevant areas, such as sea-level studies and palaeoecology, receive minimal and less than up-to-date treatment. Together with deficiencies in referencing the more advanced literature, these lacunae possibly reflect the author's own interests as well as the rapid advances in the literature concerned. Nonetheless the book's strengths outweigh its weaknesses. It contains an innovative and authoritative view of Quaternary science, and will be of great practical value to earth scientists. □

Robert J. Devoy is a Senior Lecturer in the Department of Geography, University College Cork, Ireland.

• Newly published by Unwin Hyman is *Chemical Fundamentals of Geology*, by Robin Gill, intended for geology students and others wanting to get to grips with 'geo-relevant' chemistry.

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Articles should be accompanied by a heading of 50 – 80 words written to advertise their contents in general terms, to which editors will pay particular attention. A heading (printed in italic type) is not an abstract and should not usually contain numbers or measurements. The study should be introduced in more detail in the first two or three paragraphs, which should also briefly summarize its results and implications.

Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

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GENERAL

All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

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References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963 – 65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

Submission. *All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value $5' to prevent the imposition of import duty and value-added tax (VAT).*

Penury and potential in Venezuela

Maxine Clarke

Five years ago Venezuela's currency collapsed. Since then the country's researchers have been doing science on a shoestring.

TODAY, 2 February, Venezuela acquires a new president, Carlos Andres Perez, victor of the general election held in December after a year-long campaign. He inherits a stable and, in Latin American terms, remarkably democratic political system. Since the end of the dictatorship in 1958 there has been little organized unrest, and the collapse of the guerrilla movement in the mid-1970s heralded almost ten years of stability. On 'Black Friday' in October 1983, the currency fell from about three bolivars (Bs) to the US dollar to its present thirty-five. Almost overnight, imported goods became impossible to obtain and foreign travel became prohibitively expensive.



Carlos Perez — unlikely to have a science policy.

Before 1983, oil had propped up the country virtually unaided. But when the oil prices collapsed, escalating interest rates set by international banks crippled the economy. Another 50 per cent devaluation seems to be on the way. In his previous administration (1974–1979), Perez nationalized the oil industry and ended the social unrest of the 1960s by inviting guerrilla leaders to join his government. Hopes that he will repeat the trick for the present crisis were pre-empted by the outgoing president Jaime Lusinchi, who last month announced that Venezuela will stop paying the principal on about $20,000 million of its outstanding foreign debt to international banks.

Science inevitably takes a back seat in such economically crisis-ridden times. Perez will probably appoint Jaime Requena as his science minister, but the administration is unlikely to have a science policy. Some Venezuelans think this only realistic given the economic problems. Others believe that investment in research is essential for the future, particularly in developing the nascent aluminium, hydroelectric power and plastics industries.

Although there are many excellent scientists in Venezuela, the government's organization of the research effort is a shambles. Apart from CONICIT, a body that is Latin American rather than having evolved specifically in Venezuela, there is no organized, national system for awarding grants by scientific merit. Decisions are left up to individual directors of institutes or rectors of universities. Some of them do indeed apply professional criteria in distributing the limited funds available, but the lack of accountability means that others need not be so scrupulous. 'Administrative' costs, for example, can swallow up huge proportions of an institution's budget. Unpopular individuals can fail to have their projects supported, and projects can be supported for political, rather than scientific, reasons. Most researchers who have jobs have them for life, a situation open to abuse. □

These articles are based on a recent visit to Venezuela by Maxine Clarke, Nature*'s Features Editor. Thanks are due to her host Maria Centeno, and also Carlo Caputo, Nora Castaneda, Stephen Garner-Winship, Erica Jaffe, Raul Padron and Jorge Villegas.*

The institutes: self-help is the answer

EACH of Venezuela's research institutes is responsible to the ministry that founded it. IVIC, the institute for scientific investigation, is the largest. Others are IDEA, the institute of advanced studies; INTEVEP, the oil institute; FONAIP, the agricultural institute; FII, the engineering institute; and CAICET, the Amazon research institute. Most of these do not support much basic research. CAICET, for example, is mainly involved in health programmes.

As in other South American countries, research in Venezuela is administered by CONICIT, which has a budget of some 180 million Bs (about $5 million) per year. About a third of this is available to scientists who apply for grants, another third goes on students and equipment, and another third on administration. Many scientists are critical of CONICIT for spreading these meagre resources too thinly on new programmes rather than continuing to support existing projects.

Even by its rivals IVIC is said to be the best research institute in Latin America. The problems it faces are typical of the conditions faced by scientists in post-crash Venezuela. It is under the control of the minister of health, who appoints the director, who then can appoint heads of department, distribute the annual budget as he wishes and so on. The staff at IVIC elect a new director every four years, but there have been occasions when the scientists' choice has been ignored — if the candidate belongs to the wrong political party, for example. The government ministers themselves are often ex-scientists who have decided on a political career in order to implement their ideas about what sort of science should be done, a system that leads to frustration among those researchers who feel that their careers are being directed by people who have some grand scheme in mind, rather than those who will take notice of project applications from the grass-roots level.

This frustration has resulted in fragmentation of resources. Two breakaway institutes have been set up by IVIC scientists who in the past have disagreed with the way the institute is run: INTEVEP, which was formed by members of the chemistry department, and IDEA (see below).

IVIC receives an annual budget from the government of 300 million Bs (about $8·5 million). There are 12 scientific departments, called centres, service departments and administrative offices. The institute also provides facilities such as accommodation, a kindergarten, a national guard post, a bank and that Venezuelan rarity, a post office.

In the past few years the budget has become inadequate for IVIC to function effectively — the money has to pay for 100 scientists, their support staff, equipment and running costs. So other sources of income are being sought. IVIC's director, the neuroscientist Horacio Vanegas, is involved in discussions with various international science organizations and the Andean Development Corporation (CAF), a biotechnology development organization. Shopping around in this fashion is

still alien to Venezuelans, many of whom have not come to terms with the fact that since 1983 the country is no longer relatively wealthy. Other Latin American countries, such as Brazil and Costa Rica, have set up links with international funding agencies, but oil-rich Venezuela has not, and suffers from being perceived as prosperous by the outside world.

A typical salary for an established staff scientist is about $350 a month, and the 100 researchers at IVIC have to share $1 million a year for running costs, equipment, technicians' salaries and so on. Many of the researchers have been graduate students or postdocs in universities in the United States and in Europe, and some manage to obtain funds using the contacts they have made there. Others have begun collaborative projects, enabling exchange visits. "It's a pity we aren't baseball players", said one researcher, "then there would be plenty of government money available." (Baseball is the national sport, and its teams are conspicuously well-sponsored.)

IVIC provides various courses for undergraduates from the Central University in Caracas, about 150 of whom each year go on to study for higher degrees. When IVIC first began to run PhD courses in the 1970s, the universities refused to recognize the institute's right to award the degree. IVIC's response was a typical piece of Venezuelan pragmatism; it simply renamed the degree PhSc (philosophus scientius).

Unofficial responses

The researchers themselves have developed many unofficial responses to the economic crisis in which they now find themselves. Officially, there is a preferential exchange rate for government employees of 14.5 Bs to the US dollar rather than the commercial rate of 35 Bs. But any researcher trying to obtain equipment or materials using this method will experience horrific bureaucratic delays. There are stories of how companies have gone bankrupt between receipt of an order and the payment of the cheque (stopping the process at this point is virtually impossible). There is no efficient way by which customs identifies the goods as different from commercial goods, so degradable chemicals, including isotopes, can be delayed for months. It is not surprising that smuggling is the most common way by which scientists obtain the tools of their trade. One brought in his computer with the help of a brewery, whose beer is the only brand IVIC staff will drink.

Many people have built their own equipment out of the materials they have to hand; jury-rigged modem connections are common, and one researcher had made an optical diffractometer using parts of his own camera. This same person built a rotating-anode X-ray generator out of parts ordered painstakingly slowly from a British company, and then built an office on the top of the machine.

Vanegas, caught between his frustrated scientists used to better times and an unsympathetic government, emphasizes self-help as the only way forward. For the first time in the institute's 30-year history, he is going to produce a five-year plan to identify potentially fruitful areas of research. He is particularly keen on biotechnology, both plant and medical, as an area to attract funds and one that will reap rewards in the future. He is also proud of his institute's technology centre, which provides an analytical service for which it charges. He believes the service provided cannot be bettered anywhere in Latin America, and now that industries and hospitals are unable for financial reasons to send samples for analysis outside the country, they are beginning to use the IVIC service routinely. The profits are divided equally between the institute, the laboratory that has provided a particular test and the people who perform the test. Last year, the centre made 4·5 million Bs ($130,000) profit.

But at IVIC as elsewhere in the country, science is done in an eccentric and personal way. Instead of groups of researchers working on related aspects of a problem, such as can be found in any institution in the United States or Europe, individuals work on their own project, acquiring research students and technicians, rather than collaborating with other institutions and making the best use of resources. The physics department at IVIC is an exception to the rule, mainly as a result of an attempt to bring together all Venezuelan experimental physicists into one centre. The ratio of theoretical to experimental physicists at IVIC is about 50/50, compared with 80/20 at the universities and 90/10 among graduate students. Miguel Octavio of the cold-temperature laboratory at IVIC says that only one doctorate in experimental physics will be awarded in Venezuela in the next three years, compared with about 30 theoretical degrees.

Octavio has worked on superconductivity for many years, and has been a wry observer of the explosion of the field since the breakthrough made in 1986. Because of his contacts at MIT, where he used to work, he manages to keep in touch. But to use his undoubted expertise in the field to do research on the high-critical-temperature materials, he needs money, and this is conspicuously lacking.

Despite the lack of any compelling reason to do good science — low salaries, nepotism and the difficulty of obtaining materials and information — many people are doing excellent research. This is most apparent at IVIC, where the laboratories are bustling, busy places. Many have taken a conscious decision to abstain fro the in-fighting for privilege and try to d the best research and teach as man students as possible, in preparation fo better times. But with their experience o how the country, and its science, are run few are optimistic; IVIC has lost one quarter of its staff to positions abroad i the past five years.

IDEA for change

The institute of advanced studies (IDEA stemmed from acrimony in the 1960s The then director of IVIC, Raymund Villegas, conceived the notion of an insti tute to study the development of scienc and the interface between science and th arts. When Villegas became minister o science in 1979, he persuaded the govern ment to set up a new institute, and took with him from IVIC his two brothers Jorge and Leopoldo, and one or two othe scientists.

Before the present director, the philosopher Luis Castro, took over in 1985, there were seven scientists and one researcher from the humanities at IDEA. In the past three years, Castro has appointed six more staff from the humanities: an artist, a philosopher, a political scientist and a cultural anthropologist among them. Castro wants to promote the interdisciplinary approach, and create an environment where art and science can meet. The laboratories at IDEA contain plenty of equipment but a marked absence of people to operate it — unlike laboratories in the universities, hospitals and at IVIC, where there are more people than facilities.

IDEA receives a grant of six million Bs ($170,000) a year, which Castro says is inadequate on its own to run the institute. When it was founded, at a time when the economy was in a better state, IDEA was intended to be an international conference centre, thus generating income of its own. Castro believes that conferences could still be the saving of the institute, pointing out the financial attraction now that the bolivar is so weak.

He stresses that Venezuela has much to offer scientists around the world: there are many unique natural features waiting to be studied, few regulations to restrict experimental programmes (although the bureaucracy of the government is another matter) and a great deal of scientific talent that is eager to undertake international collaboration. The researchers themselves are more sceptical. The troubled World Archaeology Congress is scheduled to hold its next meeting in Venezuela, at the University of Los Andes. But there is not much grass-roots enthusiasm for hosting an event that has become so politically sensitive. Already the manoeuvring over the organization of the congress has begun, diverting energies that would be better spent on research. □

The universities: looking to local relevance

VENEZUELA's university system is in imminent danger of collapse. From January to May last year, the system was paralysed by a strike of teachers, other staff and students. No classes were held. The cause was the government's failure to keep its promise, made in 1982, to keep staff salaries index-linked to inflation. The strike ended when the government proposed a compromise budget to be implemented last autumn; although university staffs are now back at work, they fear that the worsening economic situation will again allow the government not to keep its promises.

Yet Venezuela has an excellent tradition of university education, in principle free to all. There are 16 public universities and seven small private universities. Venezuela's 'Ivy League' are five 'autonomous' public universities, of which the most important is the Central University in Caracas (UCV), with 50,000 undergraduates. The others are the universities of Zulia, Los Andes, Caribibo and Oriente.

But free university education does not mean free access; in the absence of government loans or grants for the less well-off, higher education remains only a dream for many. Nonetheless, altogether there are more than 300,000 students in Venezuela.

The remaining public universities, set up in the 1960s, are called 'experimental' universities. They were founded at a time when there was much student activist support for anti-government guerrilla forces. At UCV the government converted all the halls of residence into classrooms, an arrangement that still persists, even though student radicalism is now limited to the vocal. The experimental universities have fewer constitutional rights than the autonomous universities, because each one is under the control of the government minister who set it up.

The universities' dispute was triggered by a request for a special loan for running expenses, which the universities were finding hard to meet. When the request was turned down, the strike began, with staff demanding the promised cost-of-living increases, which have not been provided since 1982. In the past three years the cost of living has increased by more than 40 per cent per year. Once the strike began, the students joined in, demanding an improvement in services such as libraries, transport, food and so on.

Postgraduate studies are in an even worse state than undergraduate courses. At UCV, for example, every faculty has a centre for postgraduate studies, but each one has only very few students. Most of them are studying for masters' degrees; there are only a handful of PhD students. The new rector of UCV, Luis Fuenmayor Toro, is trying to increase the numbers of masters' and doctoral students from the present two per cent of the total number of students at UCV. He also wants PhD students to be more research-based instead of class-taught, as many of them are at present.

Toro believes that one approach to the seemingly desperate economic problem is to emphasize research that is of direct relevance to the Venezuelan people. Many researchers are aware of the need to form partnerships with international colleagues, and feel that Venezuela in better times has perhaps taken a rather haughty view of the rest of South America, a luxury it can no longer afford.



Tony Morrison

A cheerful mural at the Central University in Caracas. But university staff remain dissatisfied.

The government invests about 7,000 million Bs ($200 million) per year on higher education (this figure includes teachers' training). The universities regard this as inadequate, mainly in view of the inflation of the past few years, which has not been mirrored by a corresponding increase in their budgets. The cabinet decides the annual education budget, which is then approved by congress. Once approved, the money is distributed to all the universities by the National Council of Universities. This is done on the 'night of biting' in which each university tries to get the best share. The autonomous universities generally obtain most of the money, but the experimental universities have another potential source of funds: the minister for education. It has become a tradition that the minister of education in a particular administration creates his own experimental university, with a political appointee as dean.

By far the largest and best of the experimental universities is Simon Bolivar University (USB), which supports about 6,000 undergraduate students and 1,000 graduates. The buildings and grounds are beautifully maintained, and the colonial-style architecture betrays the origins of the campus — first a coffee plantation, then in turn a farm for sugar, horses, flowers and now, the joke goes, students. It was set up 21 years ago in response to the guerrilla activities of the students at UCV. Situated well outside the city, the only public transport to civilization from USB is an irregular half-hourly bus.

The science departments concentrate on technical subjects, particularly computing studies, information technology, metal alloys, physical chemistry and food chemistry. Biology was born relatively late here, but the department has learnt to optimize its resources, according to Dr Klaus Jaffe, by taking advantage of Venezuela's wealth of natural diversity. Hence there is a palaeontology group investigating dinosaur and other fossils; and ecology, marine biology and parasitology departments (although no molecular biology department, not least because up-to-date equipment would be impossible to buy and maintain). Much of the research done here is published in the international literature.

Jaffe believes that the autonomous universities are too large for effective management, and says that the system at USB for distributing money and choosing officials is at least as free as it is in the autonomous universities. The academic staff of the university suggest three names to the government when a new dean is to be elected, and the government selects one of these names. Money awarded to the university is distributed internally according to strict criteria such as productivity, as well as in start-up grants for new researchers. The university receives about five million Bs (about $140,000) a year for research. This amount is supplemented by the energy of individual researchers, who obtain grants from overseas, particularly Sweden, Japan, Canada and the United States, as well as getting a piece of the small cake distributed by CONICIT. The foreign research money takes almost any form one cares to imagine, from Swedish 'pocket money' to full-blown grants from the National Science Foundation as part of that organization's US–Latin America research scheme. Jaffe says that this constant searching around for money is yet another handicap for scientists in the developing world struggling to keep up with the international pace. □

Segmental patterns of neuronal development in the chick hindbrain

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Identification of specific neuronal populations and their projections in the developing hindbrain reveals a segmental organization in which pairs of metameric epithelial units cooperate to generate the repeating sequence of cranial branchiomotor nerves. Neurogenesis also follows a two-segment repeat, suggesting parallels with insect pattern formation.

THE remarkable diversity of function produced by the vertebrate CNS is based on regional variations in the identity, arrangement and connectivity of nerve cells. A major aim of neurobiology is to understand how such variations develop. One possibility is that cells are organized into repeating groups, or segments, along the antero-posterior (A-P) axis, allowing each segmental population to individuate from its neighbours. Although segmentation is prominent in the development of many invertebrates[1-4], its contribution to vertebrate development is less clear cut. During this century at least, only the somitic and nephrogenic mesoderm have been regarded as being properly segmented, but it is possible that segmentation is also an important pattern-forming process within the neural epithelium, where transient periodic swellings, neuromeres, have long been recognized[5-7]. In higher vertebrates, neuromeres are particularly conspicuous in the developing hindbrain; an extensive literature has described these 'rhombomeres' in the embryos of many species, amongst which the numbers and arrangement of swellings are constant (reviewed in refs 8, 9). The original descriptions of rhombomeres culminated in two opposing views of their significance, clearly outlined in 1918 by Neal[7]: first, 'the non-phylogenetic interpretation that neuromeres are either a) artifacts due to the action of fixing agents or, b) transient embryonic structures resulting either from longitudinal compression of the neural tube or to the local strain of related nerves'; and second, 'the phylogenetic interpretation that neuromeres are the visible remnants of a primitive segmentation of the nervous system and consequently reliable clues to the original number of metameres in the vertebrate head'. There have been surprisingly few attempts to relate this gross pattern of swellings to any underlying patterns of neuronal development[7,10,11]. Some authors did suggest that there may be a correlation between the development of particular cranial nerve nuclei and their origins from individual rhombomeres[8,10,11], but others disputed such relationships[7]. This uncertainty was probably a result of limitations in the techniques available at the time. An understanding of the significance of rhombomeres will require the elucidation of their cellular basis. This is also necessary for the interpretation of the expression patterns of genes with putative regulatory roles during the regionalization of higher vertebrates. Many mouse homoeobox genes, for example, have boundaries of expression that lie within the developing hindbrain[12-17].

Here we show that the neuronal pattern of the chick hindbrain does develop on a segmental basis, and that the early arrangement of the cranial nerve nuclei reflects these segmental origins. We find, moreover, that the pattern of hindbrain segmentation shares certain features with the segmentation process described in organisms such as *Drosophila melanogaster*.

Segmental patterns

Rhombomeres arise between stages 9 and 12 and persist up to stage 24 (ref. 9; Fig. 1*a*). When the full complement of rhombomeres has been generated, the neural epithelium is subdivided into repeating units by an iterative pattern of neurogenesis. The first neurons appear within alternate rhombomeres. At stages 13–14, axons of these reticular neurons are most numerous in rhombomeres 2, 4 and 6 (r2, r4, r6), whereas neurofilament immunoreactivity in r1, r3 and r5 is mostly confined to cell bodies of newly-differentiated neurons (Fig. 1*b*, *c*). At stage 16 the alternating density of neurofilament staining is maintained by the growth of motor axons within the even-numbered rhombomeres (Fig. 1*d*, *e*). Between stages 16 and 17 this alternation diminishes as motor axons also begin to appear in the odd-numbered rhombomeres (Fig. 1*f*).

Rhombomere boundaries

Transversely oriented boundaries between adjacent rhombomeres are revealed by concentrations of axons visible in neurofilament-stained preparations from as early as stage 13 (Figs 1*b*, 2*a*). In the boundaries, large numbers of neurones differentiate beneath the segmental ridges of the ventricular surface.

As medially-directed axons approach the midline floor plate, many turn into the medial longitudinal pathway, contributing projections to other CNS regions. Such axons are particularly concentrated at the r4/5 and r5/6 boundaries (Fig. 1*b–e*). Other axons continue to grow across the floor plate before turning into the contralateral medial longitudinal pathway (Fig. 1*e*).

At the boundaries, neuronal cell bodies and axons populate embrasures underlying the ventricular ridges (Fig. 2*b*). The intercellular spaces in these regions are large compared with those in the adjacent neural epithelium (Fig. 2*c*, *d*). Moreover, they are distinguished by speckled immunoreactivity to the extracellular matrix glycoprotein laminin (Fig. 2*e*). Expression of the highly sialylated neural cell adhesion molecule N-CAM in the ventricular layer, in contrast, is localized to the central regions of rhombomeres (Fig. 2*f*). Finally, Ng-CAM/L1 is expressed at the rhombomere boundaries (Fig. 2*g*).

The boundaries between rhombomeres are therefore distinct from the central regions; the large intercellular spaces may permit axons to extend preferentially, thereby forming discrete segmented pathways across the transverse axis. The local expression of laminin suggests another way in which this process may be facilitated and Ng-CAM/L1 expression suggests that some axons fasciculate when growing in these transverse pathways.

Cranial nerve roots

The exit points of the cranial nerves occupy distinct positions in relation to individual rhombomeres (Figs 1*c*, *f*, 4). The Vth (trigeminal) nerve root, for example, lies in r2 and the VIIth (facial) nerve root lies in r4. In each case, the root lies in the central furrow of the rhombomere, between the two boundaries. Injection of the fluorescent tracer DiI into the Vth, VIIth and IXth (glossopharyngeal) roots reveals a tight correspondence between the position of each branchial motor nucleus and the

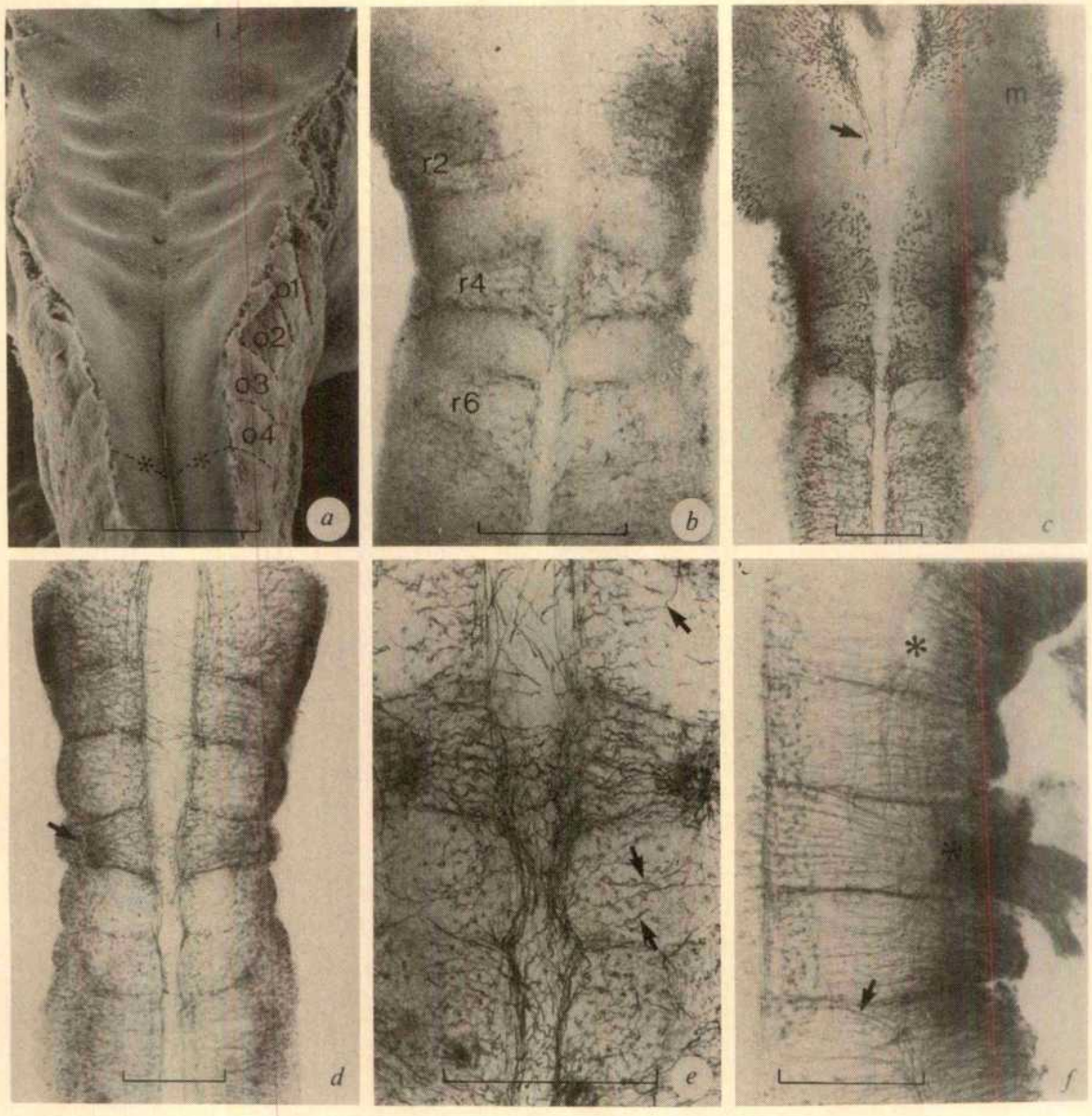


Fig. 1 *a*, Scanning electron micrograph of the chick embryo hindbrain at HH stage 17 (ref. 33). The ventricular (inner) surface of the hindbrain is exposed by removal of the roof plate. A smooth sinusoidal pattern of transverse ridges and furrows is visible along the A–P axis. The ridges do not extend into the midline floor plate. Between the midbrain/hindbrain boundary (isthmus, i) and the hindbrain/spinal cord boundary (*) there are six ridges alternating with seven furrows on each side of the midline. Rhombomeres 2–6 each comprise a furrow flanked by two half-ridges. R7 and r8 lie alongside the four occipital somites (o1–o4). Before stage 15, a ridge separates these two last rhombomeres at the level of the cleft between the first two somites[9]. Scale bar, 300 μm. Fig. 1*b–f*, Whole-mounted neurofilament-stained brainstem preparations dissected from chick embryos of stages 13–17, viewed from the pial (outer) aspect. Scale bars, 300 μm. *b*, Stage 13. Rhombomeres 2, 4 and 6 are indicated, and are distinguished from r1, r3, r5 and r7 by their greater neurofilament immunoreactivity of reticular neurons; axons are also prominent at boundaries. Some axons turn in a posterior direction at the margin of the floor plate. *c*, Stage 14. Neurons have now differentiated in r1, r3, r5 and r7, whereas many axons have appeared in the even-numbered rhombomeres. The midbrain–hindbrain boundary is seen as a sharp change in density of neurofilament staining. In the midbrain, axons are descending from the diencephalic nuclei of the medial longitudinal fasciculus (mlf, arrowed). Neurons in the mesencephalic tectum (m) are extending axons. *d*, Stage 16. The boundaries between rhombomeres contain clusters of axons, particularly at r1/2, r2/3, r3/4 and r4/5. The root of the VIIth nerve (arrowed) is visible in r4; other roots have been avulsed. The medial longitudinal pathway extends along the length of the hindbrain, adjacent to the floor plate. Commissural reticular axons enter the floor plate from the medial ends of rhombomere boundaries. *e*, Stage 16$^+$. The exit point of the VIIth nerve is visible in r4, which is packed with motor axons (compare with Fig. 3*b*). Motor axons (arrowed) are also beginning to develop in r3 (Vth nerve) and r5 (VIIth nerve). Many boundary axons lie outside this focal plane. *f*, Stage 17. Motor axons course laterally in r2 and r4 to reach the exit points (*) of the Vth and VIIth nerves. Axons of these nerves are also visible in r3 and r5, and describe an arcuate, anteriorly-directed trajectory. Axons of the IXth nerve (arrowed) originate posterior to the r5/6 boundary.

Methods. For whole-mount neurofilament staining, embryos were immersion-fixed for 24 hours in 4% paraformaldehyde in 0.1 M phosphate buffer *p*H 7.2 at 4 °C. Endogenous peroxidase was blocked by immersion in phosphate-buffered saline (PBS) +0.05% hydrogen peroxide overnight at 4 °C, followed by incubation in anti-68K neurofilament antibody (gift of Dr P. Hollenbeck) + 1.0% Triton X-100 for 4 days at 4 °C. Embryos were then incubated overnight in peroxidase-conjugated donkey anti-rabbit immunoglobulin (Amersham), followed by immersion in diaminobenzidine (DAB, Dotite, 500 μg in 1 ml Tris pH 7.3) for 3 hours at 4 °C in the dark. Hydrogen peroxide was then added and the DAB reaction product developed at room temperature. The hindbrains were then dissected from the embryos, freed of pial cells, and cut down the dorsal midline. They were then splayed out like kippers to present the entire pial surface, and mounted, without flattening, in cavity slides in 90% glycerol + 10% PBS + 0.02% sodium azide.

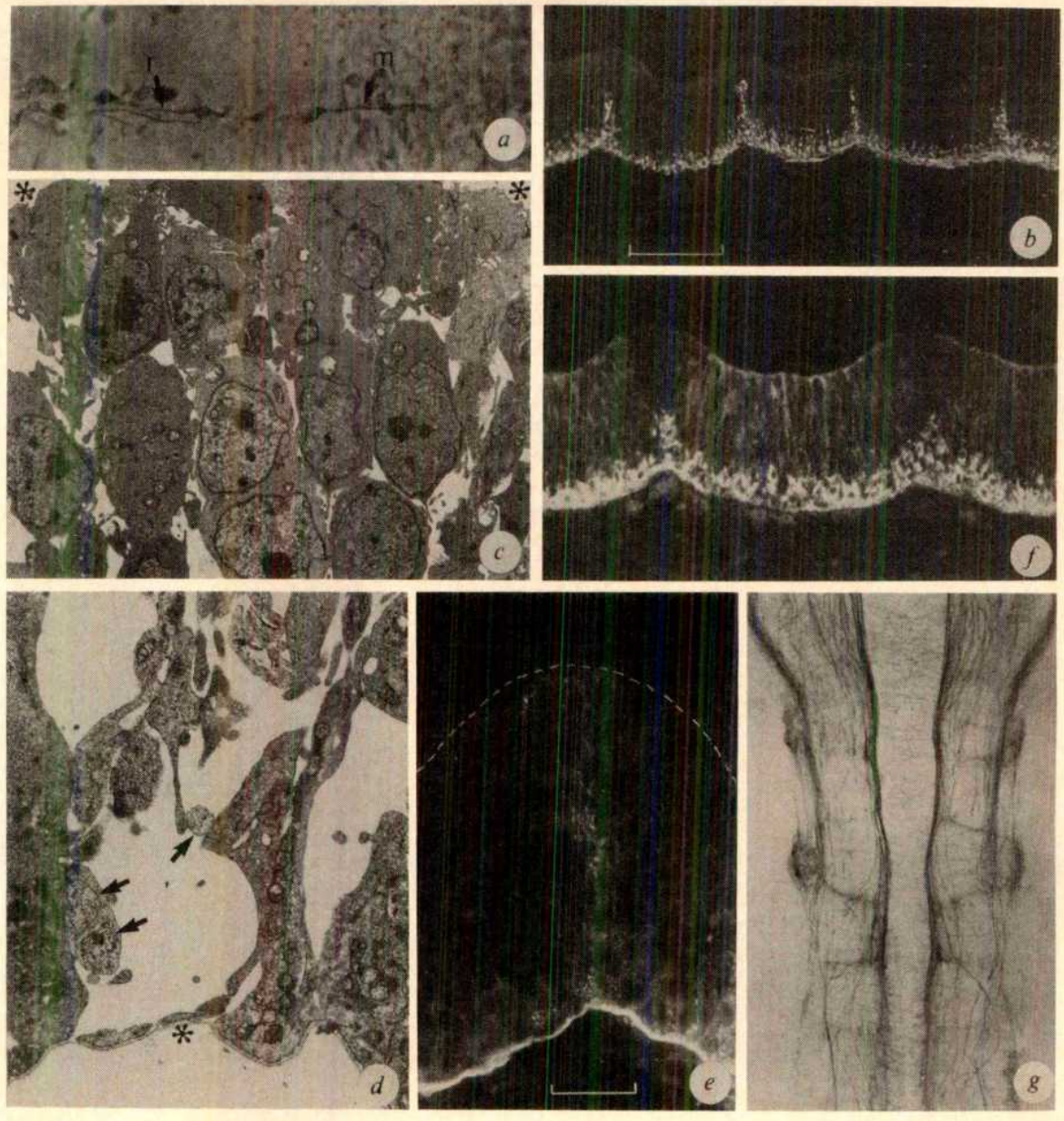


Fig. 2 *a*, Whole-mount stage-16 preparation prepared as described in the Fig. 1 legend, showing reticular (r) and motor (m) neurons lying at a rhombomere boundary. Medial is to the right. The focal plane is just beneath the ventricular surface. *b*, Parasagittal section of stage-17 hindbrain, stained by the indirect immunofluorescence method using anti-68K neurofilament antibody. At the boundary regions, the mantle layer extends towards the ventricular surface in a delta-shaped embrasure. These dorso-ventral bands of neurofilament immunoreactivity underlie the ventricular ridges. Scale bar, 100 μm. *c*, *d*, Parasagittal transmission electron micrograph of a stage 16 boundary region, showing the ventricular surface (*, *c*, ×1,890) and the pial surface (*, *d*, ×5040). The intercellular spaces are distinctly larger near the pial surface and contain several axon profiles (arrowed). *e*, Parasagittal section of stage 17 hindbrain, stained by the biotin-streptavidin amplified indirect immunofluorescence method, using affinity purified anti-laminin antibody[34] (JW1, gift of Dr J. Winter). A punctate distribution of laminin immunoreactivity is localized to the boundary region. The speckled immunofluorescence extends throughout the medio-lateral extent of each rhombomere boundary. The ventricular surface is indicated. Scale bar, 25 μm. *f*, Parasagittal section of stage 17 hindbrain, stained by the indirect immunofluorescence method, using monoclonal anti-N-CAM antibody (5A5, directed against highly sialated form, gift of Dr T. Jessell). Immunoreactivity in the ventricular layer is localised to the central furrows of rhombomeres. Neurons also express N-CAM. *g*, Whole-mount stage 17 hindbrain, stained by the modified indirect immunoperoxidase method described in the Fig. 2 legend, using monoclonal anti-Ng-CAM/L1 antibody (8D9, ref. 35 gift of Dr V. Lemmon). Axons express Ng-CAM in both longitudinal pathways and the transverse boundary pathways.

rhombomeric pattern. In a series of injections into 90 embryos, we examined how this correspondence evolves between stage 16, when motor neurons first become traceable by injections outside the brain, and stage 24, when motor neurogenesis and migration are complete.

At stage 16, motor cell bodies of the Vth nerve lie exclusively within r2 (Fig. 3*a*), those of the VIIth in r4 (Fig. 3*b*) and those of the IXth in r6. In each case, the neuronal group spans the A–P length of the rhombomere, and the axons converge towards their exit point as they course laterally in the marginal layer.

At stage 17 the number of retrogradely-labelled neurons in r2, r4 and r6 has increased, and neurons originating from the alternate rhombomeres (r3, r5, r7) are also labelled (Fig. 3*c*, *d*). In the case of each branchiomotor nerve, therefore, the first axons arise from the rhombomere containing the nerve root, whereas later axons also arise from the posteriorly adjacent rhombomere (see also Fig. 3*e*). The pathway of motor axons in the odd-numbered rhombomeres is different from that in even-

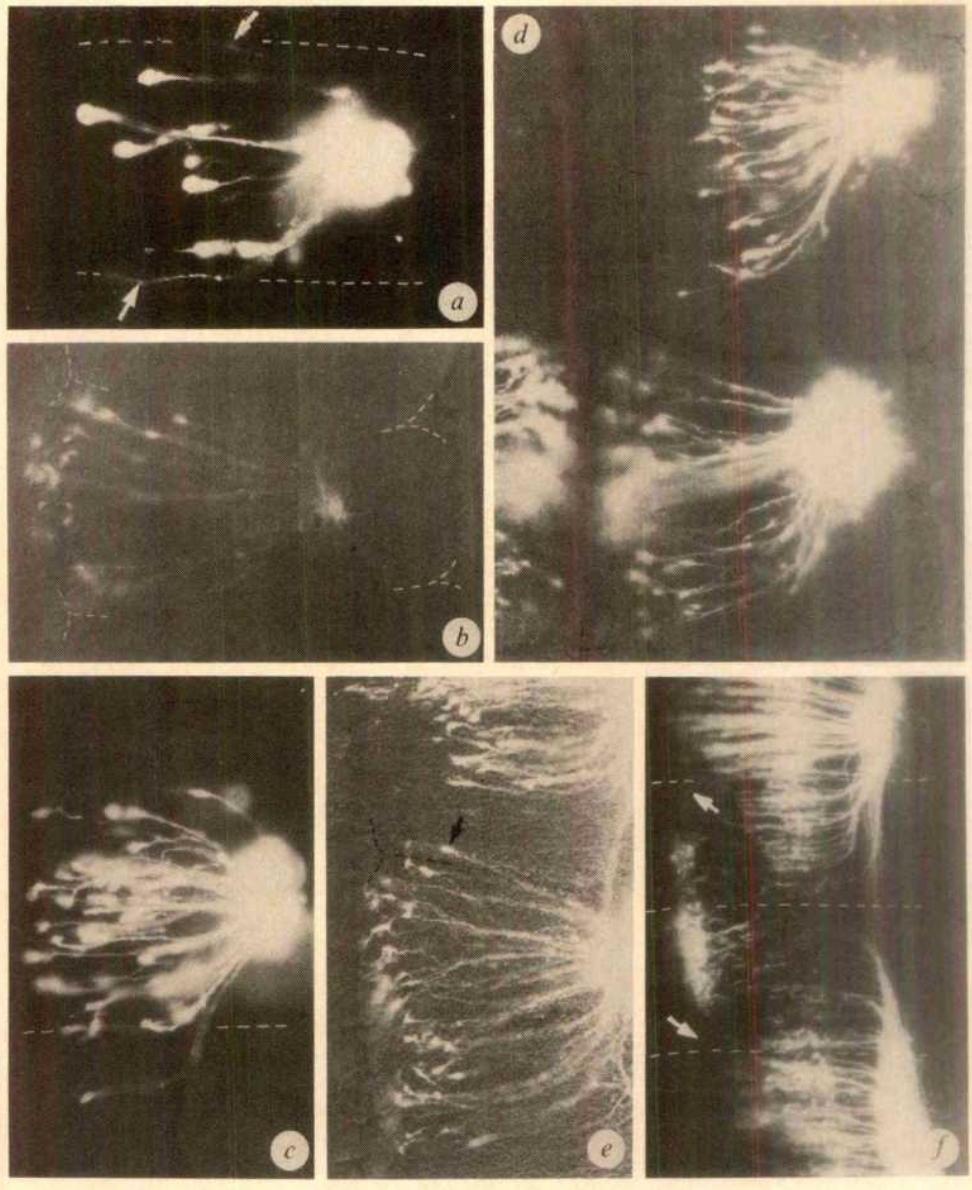


Fig. 3 Fluorescence micrographs of stage 16–19 living whole-mounted hindbrains after injection of cranial nerve roots with DiI. Anterior is to the top, lateral to the right. *a*, Stage 16. Motor neurons of the Vth nerve lie exclusively within r2. The positions of rhombomere boundaries are indicated by dashed lines; axons (arrowed) lie in both the r1/2 and r2/3 boundaries. *b*, Stage 16. Motor neurons of the VIIth nerve lie exclusively within r4. Axons lie in both the r3/4 and r4/5 boundaries; rhombomere boundaries and floor plate–basal plate boundaries are indicated by dashed lines (combined Nomarski/fluorescence optics). *c*, Stage 16+. Motor neurons of the Vth nerve are more numerous in r2, and a single neuron is labelled in r3. The r2/3 boundary is indicated *d*, Stage 17. For both Vth and VIIth nerves, neurons are labelled in two rhombomeres. The r1/2, r2/3, r3/4, r4/5 and r5/6 boundaries are indicated at the lateral edge of the neural epithelium (combined bright field/fluorescence optics). *e*, Stage 17. A single motor neuron (arrowed), labelled by injection of the VIIth root, lies anterior to the r3/4 boundary as indicated (combined Nomarski/fluorescence optics). Such exceptions to the general pattern of motor nuclear origins were observed in nine of 34 stage 17 embryos. *f*, Stage 19. Motor axons of VIth, VIIth and IXth nerves originate from distinct mediolateral as well as A–P positions. The VIth nerve nucleus (labelled by DiO, anterior and posterior limits of DiO labelling indicated by arrows) lies medial to, and is co-extensive with, the r5 component of VII and the r6 component of IX (both labelled with DiI). Sensory axons are labelled in the lateral longitudinal pathway. Boundaries r4/5, r5/6 and r6/7 are indicated.

Methods. The cranial nerve roots were exposed by dissection of the pharyngeal roof and the fluorescent carbocyanine dyes DiI or DiO[36] (Molecular Probes Inc., Junction City, Oregon, 3 mg DiI or DiO in 500 μl dimethyl formamide and 500 μl absolute ethanol) were pressure injected through a 5 μm tip glass micropipette. Embryos were then incubated for 3–4 hours in oxygenated Earle's balanced salt solution at 32 °C in a 95% O_2, 5% CO_2 atmosphere. The hindbrains were dissected from the embryos and viewed immediately under green (DiI) or blue (DiI + DiO) excitation.

numbered rhombomeres; after an early transverse trajectory, axons turn and cross their anteriorly-adjacent boundary to reach their exit point (Fig. 3*d*). Thus, motor axons cross certain boundaries (r2/3 and r4/5), but do not cross others (r1/2, r3/4 and r5/6). All of the boundaries are crossed, however, by axons descending from the midbrain in the lateral longitudinal pathway and by the ascending and descending axons of cranial sensory ganglion neurons. By stage 19, the Vth, VIIth and IXth nerves are related to a column of motor neurons, extending from the r1/2 boundary to a level opposite the first somite, in which the serially-adjacent branchial motor nuclei are segregated at the alternate rhombomere boundaries r3/4 and r5/6 (Fig. 4).

Injection of DiI into the common root of the vagus (X) and accessory (XI) nerves labels cell bodies in a region that is bordered anteriorly by the r6/7 boundary. Rhombomere 7, therefore, contributes neurons to both the IXth and Xth/XIth nerves.

Although the Vth, VIIth and IXth nerve nuclei occupy serially adjacent positions along the A–P axis, the nuclei of the somatic motor system are discontinuous. The generation of these cells conforms, nevertheless, to a segmental pattern (Fig. 4). The IVth nerve nucleus (trochlear) appears at stage 17 in r1, the VIth nerve nucleus (abducens) lies in both r5 and r6 (Fig. 3*f*) and the XIIth nerve nucleus (hypoglossal) lies in r8. It is interesting that the rhombomere pairing of the VIth nerve is out of register by one segmental unit with that of the neighbouring branchial motor nuclei.

Rhombomeres and branchial arches

The branchial nerves V, VII and IX enter the anterior part of their respective branchial arches (first, second and third). In each case, the arch lies ventral to the pair of rhombomeres in which the corresponding motor neuron cell bodies are located; the clefts between arches 1/2 and 2/3 align with the rhombomere

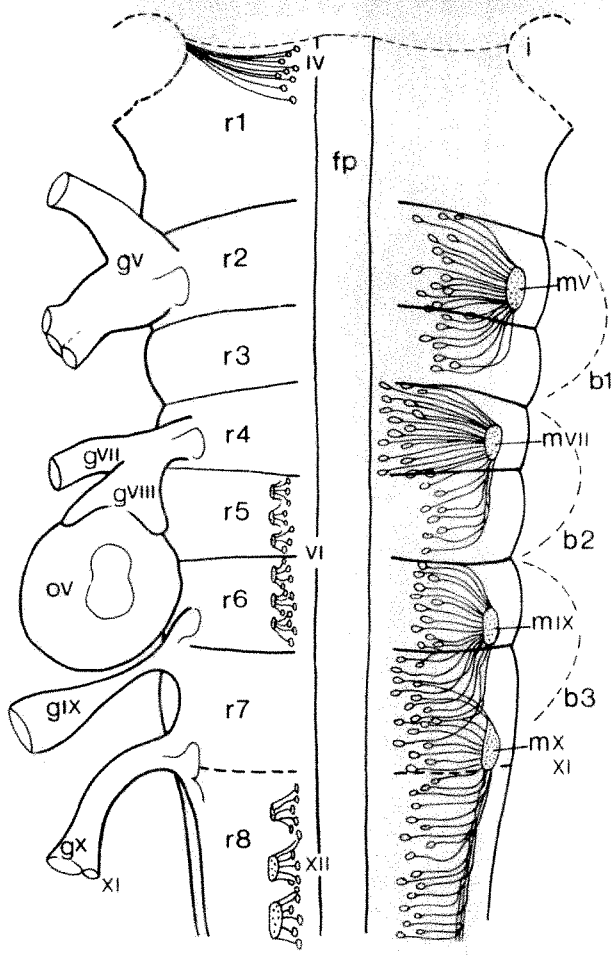


Fig. 4 Schematic diagram of the stage 21 chick embryo hindbrain, based on neurofilament-stained and tracer-injected preparations. The relationship of cranial sensory ganglia (gV–gX), branchial motor nuclei, somatic motor nuclei (IV–XII) and the combined roots of the sensory and branchial motor nerves (mV–mXI) to the rhombomeres (r1–r8) and branchial arches (b1–b3) is shown. ov, otic vesicle; fp, floor plate; i, isthmus/midbrain-hindbrain boundary.

boundaries r3/4 and r5/6 respectively. Thus each of the three principal branchial arches lies in register with the hindbrain segments, in a 1:2 ratio (Fig. 4).

Two-segment repeats

The existence of early segmented neuronal populations and segment boundary-related axons shows that the gross morphological pattern of hindbrain segments finds expression at the cellular level. The process of neurogenesis undergoes a spatiotemporal alternation in successive rhombomeres, involving both the reticular formation and the branchial motor system. Moreover, each branchial motor nucleus occupies a distinct position with respect to the rhombomere boundaries, and is constituted by neurons from two adjacent rhombomeres lying in register with the appropriate branchial arch. These patterns suggest that there are segment specification mechanisms involving two-segment repetition, as has been described for *Drosophila* segmentation[18]. With this in mind, it may be significant that a 'pair-rule'-like pattern of gene expression in the mouse embryo correlates with the alternating pattern of cellular development described here. In E9.5 embryos, the zinc-finger *Krox-20* is expressed in r3 and r5, in each case precisely within the morphological confines of the rhombomere[19].

Segmentation of the neuraxis

The nerve-cord of amphioxus[20], and the hindbrain and spinal cord of fish embryos[21–25] contain segmental arrangements of neurons, but these do not exist in the spinal cords of higher vertebrate embryos[26]. Vertebrate evolution has presumably involved a transition from a segmentally-organised neuromuscular control system for swimming to one more dependent upon long-range integration and control by higher centres. With the attendant loss of intrinsic spinal cord segmentation, the mesoderm has assumed total dominance in trunk segmentation—of skeletal, muscular and peripheral nervous systems[27–29].

The heads of higher vertebrates, however, retain units of construction, the branchial arches, whose derivatives are both anatomically and functionally distinct. Because these require independent as well as integrated control, segmental motor and sensory units are highly conserved. In contrast to the trunk, paraxial mesoderm segmentation in the head[30] is inconsequential, and the lateral plate mesoderm is also unimportant in the patterning process. Rather, it is the neural crest, with its rhombomeric origins, which is responsible for patterning the skeletal and muscular components of each branchial arch[31,32]. Construction of the head may therefore depend fundamentally on the early segmentation of the cranial neuroectoderm.

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Recombination occurs during telomere formation in yeast

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Short stretches of cloned telomeric sequences are necessary and sufficient for telomere formation in yeast as long as the sequences are present in the same orientation as they are found in vivo. *During telomere formation, DNA termini usually undergo* RAD52*-independent recombination with other DNA termini as would be predicted by models of recombination-mediated telomere replication.*

TELOMERES, the physical ends of eukaryotic chromosomes, are essential for the stable maintenance and complete duplication of linear chromosomes. The molecular ends of chromosomes from single-celled organisms to humans bear simple repeats with a defined orientation (C-A rich strand running 5′ to 3′ from the end towards the interior of the DNA molecule), most of which fit the consensus $C_{1-8}(A/T)_{1-4}$ (refs 1-3). The CA-rich strand usually contains nicks and the GT-rich strand extends into a unique terminal structure[1]. Telomere function is also conserved: natural DNA termini from the ciliated protozoans *Tetrahymena* and *Oxytricha* (which bear, respectively, C_4A_2 and C_4A_4 repeats) support the stable maintenance of linear plasmids in *Saccharomyces cerevisiae*[4-6]. In so doing, the ciliate termini are modified by addition of yeast telomeric $C_{1-3}A$ repeats[6-8].

The mechanism for replication of eukaryotic chromosomal termini is unknown. Recombination-dependent replication models[9-14] (Fig. 1) are attractive because they can account for a number of unusual properties of telomeres, such as their ability to grow and shrink[15-18] and the presence of terminal repeats and single-strand nicks, both of which would promote telomere-telomere recombination. But it is difficult to obtain support for recombination-dependent telomere replication because normally there would be no detectable consequence of terminus-terminus recombination because it involves the exchange of identical sequences located on all telomeres. An alternative model, non-templated DNA replication, became appealing with the discovery of telomerase in *Tetrahymena*[19], a ribonucleoprotein capable of extending the 3′ end of the GT-rich strand of different telomeric sequences by the addition of G_4T_2 repeats. The modification of ciliate DNA termini in yeast may also occur via such an activity.

We have determined the DNA requirements for formation of new telomeres in yeast. Our results indicate that 28 base pairs (bp) of cloned telomeric repeats, in the natural orientation and in the absence of any special terminal structure or additional sequences, are sufficient to act as a substrate for the addition of yeast $C_{1-3}A$ repeats. We have also been able to detect recombination between DNA termini, a result consistent with models of recombination-mediated telomere replication.

Cloned telomeric repeats

Linear plasmids were constructed *in vitro* and introduced into yeast by transformation. Each of the *in vitro* constructed plasmids is chimaeric in the sense that its two ends are different. The test end, whose ability to support telomere formation is assayed, carries cloned C_4A_4 repeats, the length and orientation of which are varied in individual experiments. Linear plasmids are named according to the number and orientation of test end sequences, for example YLp112CA contains 112 bp of test end sequences oriented such that the C_4A_4-strand proceeds 5′→3′ from the end to the interior of the plasmid, and YLp28GT contains 28 bp of test-end sequences oriented such that the G_4T_4-strand proceeds 5′→3′ from the end to the interior of the plasmid. The other end of the chimaeric plasmids, referred to as the control end, is a terminal restriction fragment from the extrachromosomal ribosomal DNA of *Tetrahymena* (Fig. 2*b*) that carries ~300 bp of C_4A_2 repeats and efficiently promotes telomere formation in yeast[4,5]. Because the *Tetrahymena* fragments are derived from native rDNA, they, unlike the test end, have all of the *in vivo* modifications, such as terminal foldback structures and single-strand interruptions.

Our data lead to a number of conclusions about the molecular requirements for telomere formation (Table 1). First, a test end consisting of 112 bp of telomeric sequences oriented such that the CA-rich strand proceeds 5′→3′ from the end of the molecule, is sufficient to allow the maintenance of a plasmid as a linear DNA molecule in yeast. This result is also observed when the test end carries as few as 28 bp of telomeric sequence although the percentage of transformants containing linear plasmids decreases as the length of the tract of telomeric repeats on the test end gets shorter. In contrast, test termini with 16, 10, 4 or 0 bp of telomeric sequences or termini with repeats in the wrong orientation do not support telomere formation at detectable frequencies.

'Transfer' of terminal sequences

The structure of many linear plasmids of the correct size obtained with constructs containing 28 to 112 bp of test-end sequences was examined by restriction enzyme digestion and Southern hybridization using probes specific for both plasmid termini. For example, DNA from five independent yeast transformants produced after transformation with YLp112CA was digested with *Pvu*II which cuts once in the vector portion of the plasmid to produce two fragments (Fig. 3*a*), a 2.9-kilobase (kb) fragment containing the test end, and a 7.2-kb fragment containing the control end (before addition of $C_{1-3}A$ sequences at each end). One Southern blot of the digested DNAs was probed with $(G_4T_4)_{4.5}$ which detects test-end sequences (Fig. 3*b*). As predicted, DNA from transformants containing linear plasmids (lanes 2-6) displayed hybridization to a heterogeneous band with an average size of 3.2 kb. The test end probe displayed no cross-hybridization with either yeast or vector sequences (lane 1), or with cloned C_4A_2 repeats (lane 7). The same digestion mixtures run on an identical gel were hybridized to the $(G_4T_2)_7$ probe, which detects the C_4A_2 repeats at the control end of the plasmid (Fig. 3*c*). DNA from all transformants showed the expected band of hybridization at 8.3 kb. Again, no cross-hybridization between the $(G_4T_2)_7$ probe and either yeast or vector sequences (lane 1), or cloned C_4A_4 repeats (lane 8) was

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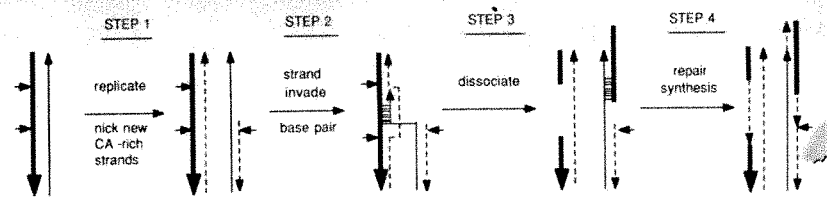


Fig. 1 Recombination model for telomere replication (redrawn from ref. 14). Each strand of a DNA duplex is represented by a line: thin lines indicate the GT-rich strand, thick lines indicate the CA-rich strand, dashed lines indicate newly replicated DNA. The 3′end of each strand is indicated by an arrowhead. Nicks in CA-rich strands are indicated by horizontal arrows. Step 1: after DNA replication and subsequent removal of the RNA primer, a 5′ terminal gap is produced on each duplex. (For simplicity, only one such gap on a pair of sister chromatids is shown.) Specific nicks are made on the newly-replicated CA-rich strand. Step 2: the free 3′ tail (thin line) invades the sister duplex, base-pairs in an out-of-register configuration with the complementary strand (thick line), displacing its complementary strand (dashed line). Step 3: dissociation of the newly base-paired region is facilitated by nicks present on the CA-rich strand of the donor (thick line). Step 4: the resulting internal gap and terminal 3′ gap on each duplex is filled by repair synthesis (dashed line) and remaining nicks are sealed by DNA ligase. Replication by this mechanism results in two fully replicated duplex molecules, one of which (duplex on the right) is longer (thereby providing a molecular explanation for how telomeres can increase in length) and has sequence information obtained from the thick duplex on the left.

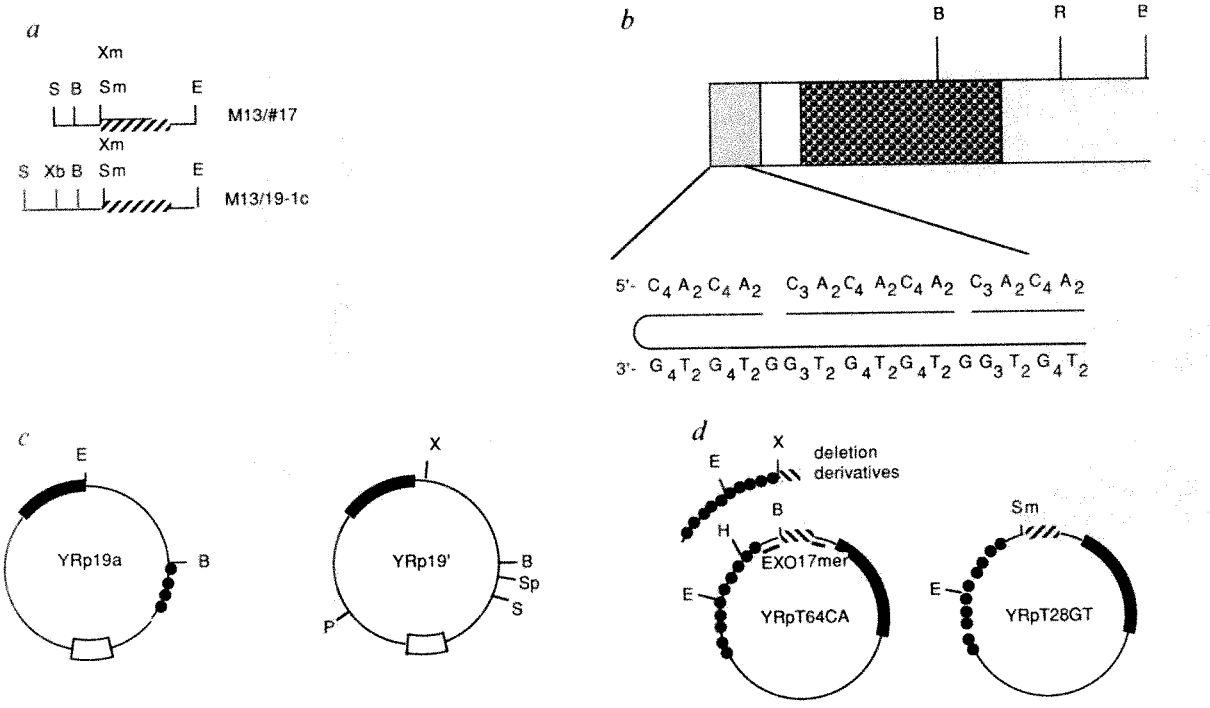


Fig. 2 DNA Structures. *a*, Cloned progenitors of test ends. The ~130-bp *Sal*I–*Eco*RI restriction fragments from either M13/#17 (mp9) or M13/19-1c (mp19) were used to create test ends of various lengths and orientations. Both plasmids contain 112 bp of 5′$(C_4A_4)_n$3′, indicated by the diagonally-striped box, where *n* was determined to be 14 by DNA sequence analysis. *b*, Control end. The terminal *Bam*HI (B) and *Eco*RI (R) restriction fragments of *Tetrahymena* rDNA were used as control end sequences on the chimaeric linear plasmids. The end of each consists of ~300 bp of C_4A_2. Adjacent unique sequences are indicated by the spotted box. Ribosomal DNA was isolated from *Tetrahymena thermophila* strain C3V (ref. 30). *c*, Vectors used to create YLp112CA, YLp112GTS and YLp112GTX. YR19′ is a 9.1-kb plasmid that contains *TRP1ARS1* (solid box) and *URA3* (open box). It was derived from YCp19 (ref. 31) by removal of the 1.5-kb *Xho*I fragment containing *CEN4*. YLp112CA was constructed by ligating test end sequences derived from M13/19-1c to the *Sal*I site and control-end sequences to the *Bam*HI site. YRp19a, which was used to construct YLp112GTS and YLp112GTX, is an 11.2-kb derivative of YRp19′ in which the 187-bp *Bam*HI–*Sph*I portion was replaced with a 2.3-kb *Bam* HI–*Sph*I fragment of λ DNA (indicated by solid circles). *d*, Vectors used to construct YLps with test ends of 64 or fewer base pairs. The important feature of these vectors is that they contain test-end sequences in either of two orientations which are exposed as a free DNA terminus upon digestion with *Bam*HI (YRpT64CA), *Xho*I (deletion derivatives of YRpT64CA) or *Sma*I (YRpT28GT), and a single *Eco*RI site for ligation of control-end sequences. *Symbols*: solid box, *TRP1ARS1*; open box, *URA3*; solid circles, λ DNA; ////, 5′$(G_4T_4)_n$3′; \\\\, 5′$(C_4A_4)_n$3′; ————, pBR322 or pUC. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hpa*I; Hd, *Hin*dIII; P, *Pvu*II; S, *Sal*I; Sm, *Sma*I; *Sp*, *Sph*I; X, *Xho*I; Xb, *Xba*I; Xm, *Xma*I.

Methods. *a*, M13/#17 was constructed by D. Gottschling by annealing two oligonucleotides, 5′-$C_4A_4C_4A_4$-3′ and 5′-$G_4T_4G_4T_4$-3′, such that out-of-register concatemers with four-base overhangs were produced. The unfractionated product was treated with T4 DNA polymerase to create blunt ends, and inserted into the *Sma*I site of M13mp9 such that the (+) strand of the phage DNA is the G_4T_4 strand. Plasmid 19-1c is the *Eco*RI–*Bam*HI fragment of M13/#17 inserted into *Eco*RI–*Bam*HI-digested M13mp19. Sequencing by the dideoxy chain termination method on alkaline-denatured[28] supercoiled plasmid templates was essentially as described[29] except that elongations were performed at 45 °C. *b–d*, For plasmid YRpT64CA, 130-bp *Sma*I–*Eco*RI fragment containing ~14 C_4A_4 repeats was isolated from M13/#17, treated with 5U S1 nuclease for 30 minutes at room temperature, and inserted into the *Sma*I site of pUC19. For plasmid YRpT28GT, the *Bam*HI–*Eco*RI fragment of M13/#17 was inserted into *Bam*HI–*Eco*RI-digested pUC19. Subsequent steps of the constructions were identical for the two plasmids. The *Eco*RI site in the polylinker was destroyed by treatment of *Eco*RI-digested DNA with T4 DNA polymerase and dNTPs to create blunt ends, followed by self-ligation. Termini of the 1.45-kb *Eco*RI fragment containing *TRP1ARS1* were rendered flush by treatment with T4 DNA polymerase, and inserted into the T4 polymerase-treated *Nar*I site of each plasmid. A 3-kb *Sph*I fragment of λDNA, which contains a single *Eco*RI site, was inserted at the *Sph*I site in the polylinker of each plasmid. The resulting plasmids, YRpT64CA and YRpT28GT, are ~7.1 kb in length, contain unique *Bam*HI and *Eco*RI sites, and retain 64 bp of 5′(C_4A_4)3′ and 28 bp of 5′(G_4T_4)3′, respectively. Position of oligonucleotides (EXO and 17-mer) used for hybridization probes and sequencing primers are indicated. Deletion derivatives of YRpT64CA were constructed by limited digestion with Exonuclease III (ref. 32) from the single *Hpa*I site in the λ DNA. Blunt ends were created by treatment with T4DNA polymerase and dNTPs. The DNAs were then ligated to *Xho*I linkers, and following *Xho*I digestion, gel purification and self-ligation, were used to transform *E. coli*. The resulting transformants were subjected to three successive screens to identify plasmids with fewer than 64 bp of C_4A_4 as well as the necessary restriction sites for construction of chimaeric linear plasmids: (1) sequential colony hybridizations[33] with end-labelled EXO III probe and with the M13 17-mer sequencing primer, (2) DNA was prepared from colonies that hybridized to the 17-mer but not the EXO III oligonucleotide probe and analysed for the presence of both the *Xho*I and *Eco*RI sites, and (3) plasmids containing both an *Xho*I and *Eco*RI recognition site were sequenced to determine the number of C_4A_4 repeats.

bserved.

Some unexpected results were obtained with both the control-nd and the test-end probes. In DNA from four of the five ransformants (Fig. 3*c*, lanes 3–6), the control-end probe also ybridized to a heterogeneous fragment with an average size of 2 kb, the same size fragment detected when the DNA samples ere hybridized with the $(G_4T_4)_{4.5}$ probe (Fig. 3*b*). These results idicate that the test end on four of the five plasmids contains $_4A_2$ repeats in addition to the expected C_4A_4 repeats. The ossibility that these results are due to ligation of the *Tetrahymena* terminus to both ends of the plasmid was eliminated by emonstrating that only the C_4A_2 repeats and none of the djacent unique DNA in the *Tetrahymena* fragment hybridizes o the 3.2-kb fragment (Fig. 3*d*). In addition to the unexpected esults with the control-end probe, in DNA from one of the ransformants, the test-end probe unexpectedly displayed ybridization to a fragment of 8.3 kb (Fig. 3*b*, lane 6) indicating hat the control end of this plasmid bears C_4A_4 as well as C_4A_2 epeats. Transfer of control-end sequences to the test end has een observed for the majority (90 per cent) of correct sized near plasmids regardless of the length of the test end (Table). On 20 per cent of the plasmids examined, C_4A_4 repeats were etected on the end of the plasmid bearing the control terminus.

The terminal location of the telomeric sequences was estab-ished by monitoring their disappearance after digestion with he exonuclease BAL-31. BAL-31 digestion was carried out on otal DNA from a strain carrying YLp112CA-5, a linear plasmid n which C_4A_2 sequences were detected on both the control and est ends of the plasmid (Fig. 3, lane 5). After BAL-31 digestion, he DNA was digested with *Xho*I which produces a 3.1-kb ragment containing the control end and a 7.0-kb fragment ontaining the test end (before $C_{1-3}A$ addition). *Xho*I digestion f yeast chromosomal DNA produces a heterogeneous fragment f ~1.3 kb derived from Y′-bearing telomeres, as well as many ther telomeric and non-telomeric fragments[7]. Figure 4 shows hat the transferred C_4A_2 repeats, like the other telomeric equences, are BAL-31 sensitive and therefore close to the lasmid end.

Total yeast DNA from a strain containing YLp112CA-5 (Fig. , lane 5) was digested with *Bgl*II (which removes the control nd, Fig. 1*c*), self-ligated and used to transform *Escherichia coli*. subclone carrying the test end of YLp112CA-5 was digested ith *Bam*HI and then for increasing amounts of time with AL-31 to determine the order of the telomeric sequences rela-ve to the end of the plasmid (Fig. 5). Because the relevant *Bam*HI site in YLp112CA-5 is in vector DNA immediately djacent to the test end, those sequences closest to the physical nd of YLp112CA-5 should be the most resistant to the exo-uclease. This analysis demonstrates that $C_{1-3}A$ sequences are he most distal sequences on YLp112CA-5 followed by the ransferred C_4A_2 repeats and then the C_4A_4 repeats, a result onfirmed by DNA sequencing (S.-S. Wang, unpublished data). herefore, transfer of control-end sequences probably occurs efore $C_{1-3}A$ addition.

What is the mechanism by which the terminal sequences are ransferred between DNA termini? The transfer is not a ligation rtefact because it involves only terminal CA-rich sequences, ot the adjacent unique sequence DNA (Figs 3, 4). Transfer annot be the result of the asymmetric resolution of a circular ntermediate formed by end-to-end fusion of the starting plasmid n yeast to give a circular plasmid with chimaeric inverted epeats[20]: asymmetric resolution of such a plasmid produces a NA terminus carrying terminal sequences in the reverse rientation, which cannot function as a telomere in yeast (Table). Finally, if telomerase exists in yeast it would be expected to dd $C_{1-3}A$ repeats, but not non-yeast telomeric repeats[19]. Therefore, the most reasonable interpretation of these data is that uring telomere formation, DNA termini usually undergo ecombination.

Table 1 Formation of linear plasmids

Plasmid	Per cent correct	Number analysed	Number transferred
YLp112CA	56	48 (13)	12/15
YLp64CA	35	20 (11)	7/7
YLp48CA	22	23 (3)	5/5
YLp40CA	9	35 (12)	4/4
YLp28CA	6	64 (42)	3/3
YLp16CA	0	148*	NA
YLp10CA	0	275*	NA
YLp4CA	0	264*	NA
YLp0CA	0	94	NA
YLp112GTX	0	54	NA
YLp112GTS	0	81*	NA
YLp28GT	0	218†	NA
YLp112CA/*rad52*	50	8† (100)	3/4

Each yeast linear plasmid (YLp) is defined by the number of base pairs and the orientation of the test-end sequences it carries (see text). The terminal structure of the test end is the DNA terminus generated by digestion with the following restriction enzymes: *Eco*RI (YLp112CA), *Bam*HI (YLp64CA), *Xma*I (YLp112GTX), *Sma*I (YLp112GTS, YLp28GT), *Xho*I (all other YLps). YLp112CA was constructed by simultaneous ligation of the 8.8-kb gel-purified vector (*Bam*HI–*Sal*I digested YRp19′) with the gel-purified 130-bp *Eco*RI–*Sal*I fragment isolated from 19-1c containing $(C_4A_4/G_4T_4)_{14}$ and with the gel-purified 1.3-kb terminal *Bam*HI fragment from *Tetrahymena* rDNA (see Fig. 2). All other YLp(n)CA plasmids were constructed by double digestion of YRpT64CA (or deletion derivatives thereof) with *Eco*RI and with the enzyme indicated above to expose test end sequences. The resulting fragment (~5.8 kb) was gel-isolated and ligated to gel-isolated 2.6-kb terminal *Eco*RI fragment from *Tetrahymena* rDNA. In most experiments, ligation mixes were introduced into strain 34c (*MATa met2, his3∇-1, leu2-3, leu2-112, tripl-289, ura3-52, cir*^{0}) by lithium acetate transformation. For the *RAD52* experiments, DNA was introduced into strain XS95-6C (*MATα, rad52-1, ura3-52, trpl, his3∇-1, leu2-112, 3*; from D. Schild) by spheroplast transformation. All of the *rad52* transformants containing linear plasmids retained hypersensitivity to X irradiation. Total undigested DNA isolated from transformants was analysed for the presence of plasmid sequences by Southern hybridization with ^{32}P-labelled pBR322. The percentage of transformants analysed which contained linear plasmids of the predicted size and structure is indicated by per cent correct. Other classes of transformants were those containing circular plasmids, no plasmids (that is gene conversion), or plasmids larger than 23.7 kb most of which did not hybridize to the test-end probe. When control termini are ligated to both ends of the vector, the per cent correct is similar to results obtained with YLp112CA. Unless otherwise noted, values reflect data collected from a single experiment. For constructs producing no linear plasmids of the correct size, nearly every transformant was analysed. For those constructs which produced linear plasmids of the correct size, the percentage of the total number of transformants which were analysed is indicated in parentheses (under number analysed). The number of linears of correct size that displayed transfer of terminal sequences over the number of linears of correct size examined is presented (number transferred). NA, not applicable.

* Data pooled from two independent experiments.

† Data pooled from three independent experiments.

The *RAD52* gene product is required for the majority of mitotic recombination events, including chromosome 'healing' by gene conversion[21]. The *RAD52* product is not required for sister chromatid exchange[22,23], nor for the addition of $C_{1-3}A$ repeats on ciliate DNA termini[21,24]. When the linear plasmid YLp112CA was introduced into a *rad52* strain, three of the four linears obtained exhibited transfer of C_4A_2 repeats to the test end (Table 1). Therefore, this telomere–telomere recombination must occur via recombination between the very ends of DNA molecules rather than by exchange of internal CA-sequences that would involve *RAD52*-dependent double-strand break repair.

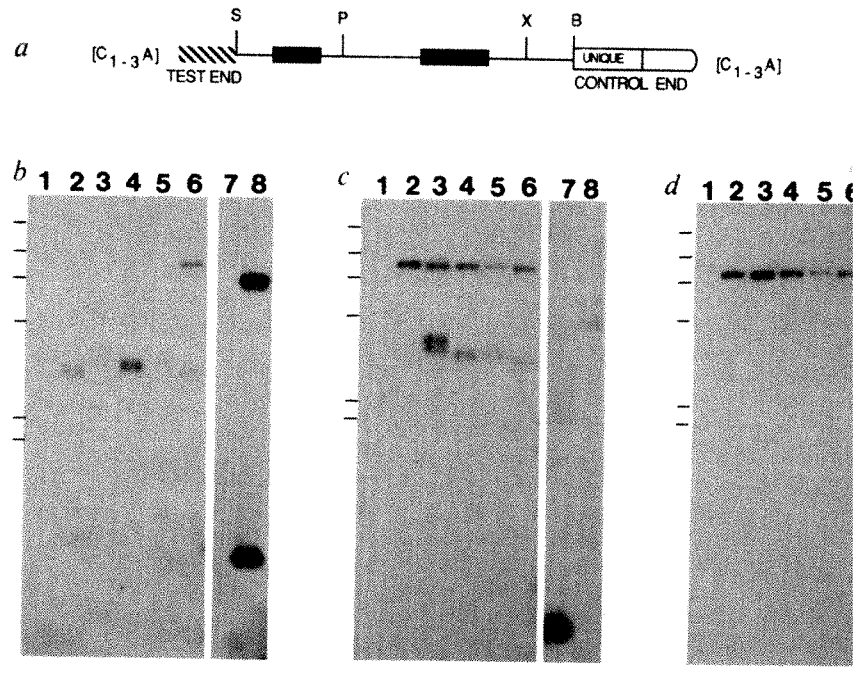


Fig. 3 Transfer of terminal sequences on a chimaeric linear plasmid. *a*, Predicted structure of YLp112CA. The linear plasmid has a predicted size of ~10 kb and a single *Pvu*II site which, when cut, produces a 2.9 kb fragment containing the test end and a 7.2 kb fragment containing the control end (before $C_{1-3}A$ addition, which is expected to add ~500 bp per terminus). *b–d* *Pvu*II digestions of total DNA isolated from transformants containing linear and circular plasmids. *b*, Hybridization with $(G_4T_4)_{4.5}$, the test-end probe, and *c*, $(G_4T_2)_7$, the control-end probe. *d*, The probe was removed from the blot shown in *c* and the filter was rehybridized with the unique sequence probe for the control end. Lanes 1–6 contain, respectively, total DNA from yeast transformants carrying YRp19′, YLp112CA-1, YLp112CA-3, YLp112CA-4, YLp112CA-5 and YLp112CA-6. Controls are lane 7, pTC5 (obtained from A. Murray), digested with *Xho*I and *Bam*HI to give a fragment (~300 bp) containing $(C_4A_2)_{50}$; lane 8, p0f1 (cloned *Oxytricha* macronuclear rDNA (ref. 34)) digested with *Pst*I to give three fragments, two of which (a 6.7-kb fragment and a 0.9-kb fragment) contain 30–35 bp (C_4A_4). Size markers indicated by horizontal lines are *Hin*dIII-digested lambda DNA.

Methods. Total yeast DNA was isolated[35], fractionated on two identical but separate gels and transferred to nitrocellulose. Hybridizations using end-labelled oligonucleotide probes were performed at 42 °C in 50% formamide, 5 × SSC, 1 × Denhardts[33], 1 mg ml^{-1} heat-denatured herring sperm DNA and 10% dextran sulphate for 8–15 hours. Hybridization conditions using nick-translated probes are described[36]. Nitrocellulose filters were washed at room temperature for 30 min in 5 × SSC, followed by a 50 °C wash for 1 h in 5 × SSC, 0.1% SDS. The 1,050-bp *Hin*dIII fragment isolated from pRP7 (ref. 37) which contains the non-coding region near the end of the *Tetrahymena* rDNA molecule was subcloned into M13mp19 and used as the unique sequence probe for the control end.

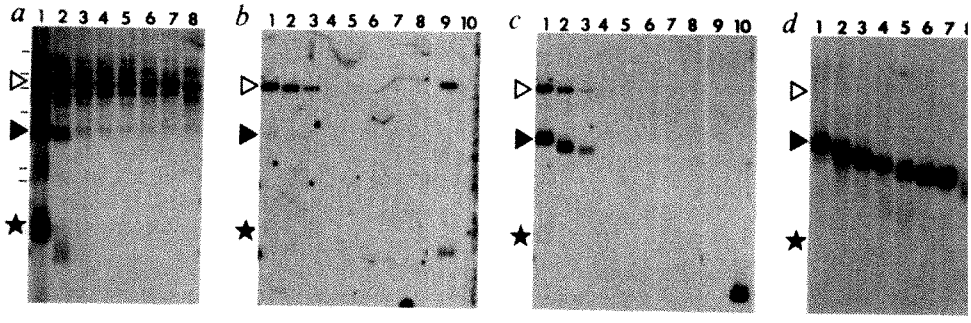


Fig. 4 Transferred terminal sequences are sensitive to BAL-31 digestion. Total DNA from a strain produced by transformation with the linear plasmid YLp112CA-5 (Fig. 3, lane 5), was treated with BAL-31. Probes are $C_{1-3}A$ (*a*); $(G_4T_4)_{4.5}$ (*b*); $(G_4T_2)_7$ (*c*) and control-end unique sequence (*d*). Lanes 1–8 contain, respectively, DNA removed after 0, 10, 20, 30, 40, 50, 60 and 120 minutes of BAL-31 digestion; lane 9, *Pst*I-digested p0f1 (contains C_4A_4 sequences); lane 10, *Xho*I-*Bam*HI digested pTEC1.6 which yields a fragment of ~700 bp containing $(C_4A_2)_{50}$. Size markers indicated by horizontal lines are *Hin*dIII-digested lambda DNA. The open arrow points to fragments carrying the test end, the closed arrow to fragments carrying the control end, and the star to terminal fragments from Y′ bearing chromosomal DNA molecules.

Methods. Samples were taken over a 2-h period, digested with *Xho*I, subjected to electrophoresis and sequential Southern hybridization. The $C_{1-3}A$ probe was prepared by gel-isolation of a 125 bp fragment which contains 81 bp of cloned $C_{1-3}A$ sequences derived from a yeast telomere[8].

Discussion

Using linear plasmids in which each terminus is marked with a different telomeric sequence, we have shown that cloned telomeric sequences 28 bp or longer support telomere formation in yeast. Although termini as short as 28 bp can support telomere formation, the number of correct sized linear plasmids recovered is small (≤9 per cent) with test ends shorter than 48 bp (Table 1). During formation of new telomeres, telomeric sequences usually undergo recombination by a process that does not require the *RAD52* gene product. Thus, although C_4A_4 and C_4A_2 repeats share only limited homology, these sequences are presumably capable of base pairing *in vivo*, possibly facilitated by the ability of these sequences to adopt unusual DNA conformations[25] and/or by a RecA-like protein that promotes strand exchange[26]. Precedent for base pairing of telomeric repeat sequences with nonhomologous but related DNA sequences is provided by the ability of poly(dGT) to hybridize under high stringency conditions to yeast $C_{1-3}A$ sequences[6,7].

What is the *in vivo* role for this telomere-telomere recombination? Recombination could be a consequence of repair or maintenance processes although its *RAD52*-independence argues against this interpretation. Alternatively, the telomere-telomere recombination may be linked to telomere formation in which case it might also be the mechanism by which $C_{1-3}A$ repeats are added to ciliate termini. Telomere recombination can be visualized in terms of a model for telomere replication[14] (Fig. 1): after replication the 3′ overhang produced by primer excision at the end of the chromosome is able to strand invade and base pair with a complementary region on another strand. In our system, this recombination could occur between the two ends of the same plasmid or between termini on different DNA molecules of these multicopy plasmids. Subsequent strand dissociation is

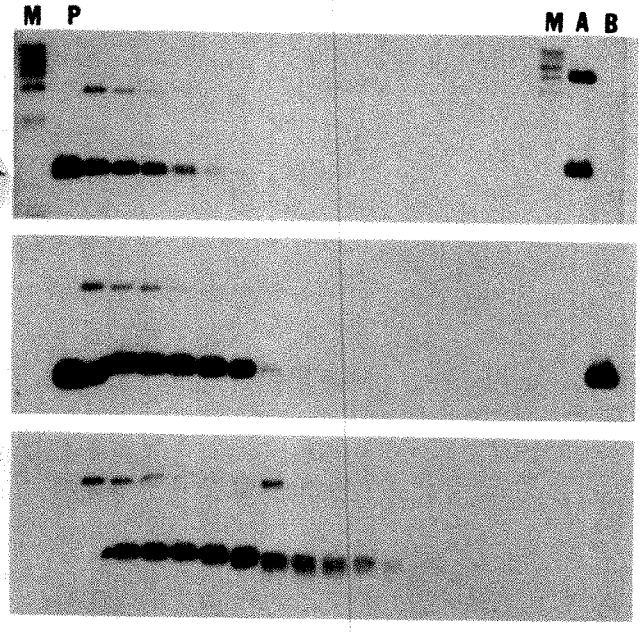


Fig. 5 BAL-31 digestion of cloned telomere. A recombinant DNA plasmid containing the test end of the yeast propagated plasmid YLp112CA-5 (Fig. 3, lane 5) was digested with BAL-31 to determine the order of the telomeric sequences. Southern blots were probed successively with: $(G_4T_4)_{4-5}$, *a*; $(G_4T_2)_7$, *b*; and $C_{1-3}A$, *c*. The lanes contain: M, *Hin*dIII-digested λ DNA; P, *Hin*dIII-*Bam*HI digested tc-0-6; A, *Pst*I-digested p0f1 DNA (C_4A_4), and B, *Xho*I-*Bam*HI digested pTEC1.6 DNA containing $(C_4A_2)_{50}$. The rest of the lanes from left to right contain samples digested for increasing amounts of time with BAL-31.

Methods. Total DNA from yeast containing YLp112CA-5 was digested with *Bgl*II, treated with T4 DNA polymerase in the presence of dNTPs and self-ligated. Digestion of YLp112CA with *Bgl*II removes the control end terminus (Fig. 2*c*), and self-ligation produces a plasmid (called tc-0-6) containing test end sequences. Plasmid tc-0-6 was first digested with *Bam*HI (the *Bam*HI site is derived from the poly-linker of M13/19-1C and is <10 bp from the C_4A_4 repeats) and then digested with BAL-31. Aliquots were removed at 5-min intervals during the first 0–30 minutes of digestion and then at 2-min intervals thereafter. Samples were digested with *Hin*dIII to release a fragment of ~1kb containing the sequences from the test-end of YLp112CA, and subjected to electrophoresis. A portion of the nitrocellulose (containing *Hin*dII-*Bam*HI digested tc-0-6 and the zero time point) was lost during washing from panel *c*. In each plasmid lane, the upper band is partially digested plasmid.

facilitated by specific nicks in the CA-strand. Any remaining gaps are closed by repair synthesis. Recombination by this mechanism is expected to be *RAD52*-independent because it does not involve repair of double-strand breaks. If recombination is necessary for telomere formation, the frequency of telomere formation is expected to be related to the length of the recombining end. Consistent with this, as the test end is decreased in size, the efficiency of telomere formation drops (Table 1). However, the fraction of plasmids of the correct size on which we detect transfer of terminal sequences is independent of the length of the test end (Table 1). Because nicks are concentrated in the CA-strand[1], the orientation requirement can also be explained by a recombination model. With telomere sequences in the reverse orientation, the invading strand is now the CA-strand instead of the GT-strand, and dissociation of the base-paired segment would require additional enzyme activities. The fact that nicks are concentrated in the CA-strand could also explain why we more often detect transfer of C_4A_2 repeats to the test end: the test end lacks single-strand nicks and therefore is expected to be an inefficient donor compared to the control end. Alternatively, the shorter test end may be an inefficient donor of terminal sequences because it (1) offers a smaller target for base pairing with the invading strand or (2) the amount transferred from this shorter tract is usually too little to be detected by hybridization.

We have demonstrated that during telomere formation in yeast, DNA termini often undergo recombination with other DNA termini. This system is the first in which there is a detectable consequence of recombination between the very ends of DNA molecules that can be distinguished from the consequences predicted by telomerase-mediated DNA replication. The question remains as to the *in vivo* role, if any, of telomere-telomere recombination. For example, the role of recombination among the terminal repeats may be to maintain their homogeneity[27]. Alternatively, recombination might be involved in telomere replication, either in concert with telomerase or not. The ultimate test of these possibilities awaits the identification and characterization of mutants defective in maintenance of linear chromosomes. If recombination is important for telomere replication, some of these mutations are likely to be in genes encoding recA-like proteins, which like the T4 uvsX protein[11], promote synapsis between terminal sequences.

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Search for strange matter by Rutherford backscattering

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According to a number of suggestions, stable strange matter could exist in the form of supermassive nuclei (or 'strange nuggets')[1,2]. In contrast to ordinary nuclei, which contain only 'up' and 'down' quarks, a piece of strange matter should comprise a mixture of 'up', 'down' and 'strange' quarks in roughly equal proportions. Small amounts of strange matter could have survived from the early stages of the Universe[1]. Alternatively, strange matter might reach the Earth as a flux of strange nuggets produced in collisions of neutron stars[3]. Limits to the cosmic flux of strange nuggets with masses in the range from 10^{-4} to 250 g have been obtained in a search for light produced by the nuggets in the upper atmosphere[4]. Here we report the results of a search for supermassive nuclei by using Rutherford backscattering of heavy ions. The method is sensitive to a broad range of masses extending to those that exceed the projectile mass by several orders of magnitude. Upper limits for the abundance of strange nuggets with masses $A \approx 4 \times 10^2$ to 10^7 AMU relative to the number of nucleons were found to be in the range 10^{-10} to 10^{-14}.

To set a useful sensitivity limit for experiments designed to search for strange matter, De Rújula and Glashow estimated the maximum concentration of strange nuggets in the Earth's crust[3]. They assume a value of $\sim 10^{-24}$ g cm^{-3} for the local dark matter density of our Galaxy. Assuming that the whole of dark matter consists of strange nuggets, this flux corresponds to an annual Earth infall of strange matter of $\sim 10^9$ g. Over the Earth's 4.6-Gyr history this results in a maximum crustal concentration of 10^{-7} by mass. For the mass range considered in our experiment, $A \approx 4 \times 10^2$ to 10^7, this limit extends from 3×10^{-10} down to 10^{-14} nuggets relative to the number of nucleons. Because nuggets with these masses are already stopped in the Earth's atmosphere[3] they should not be distributed equally over all of the crust but should be concentrated in its upper layer, in which region the local abundance of nuggets may therefore be higher by two to three orders of magnitude.

In our experiments natural samples were irradiated with beams of ^{238}U and ^{208}Pb nuclei provided by the heavy-ion accelerator UNILAC in Darmstadt. The bombarding energy was 1.4 MeV per nucleon; this is far below the Coulomb barrier of nuclei, so that the cross-section for nuclear reactions is negligible[5], and the interaction of nuclei is dominated by elastic scattering. In this case the angle and the velocity of the scattered projectile nucleus are determined by the mass of the target nucleus. In a single scattering event a projectile can be scattered into the backward hemisphere only by a heavier nucleus. Therefore, scattering of ^{238}U or ^{208}Pb through angles of 90°–180° would indicate the presence of more massive and possibly strange nuclei.

Scattered nuclei were detected by a system of twelve low-pressure multi-wire proportional counters[6] (Fig. 1) divided into four identical modules, each of which consisted of one start- and two stop-detectors. The ranges of scattering angles covered by this system are 92°–120° and 127°–170°, with a total solid angle of 1.15 sr.

The identification of detected nuclei is based on the following

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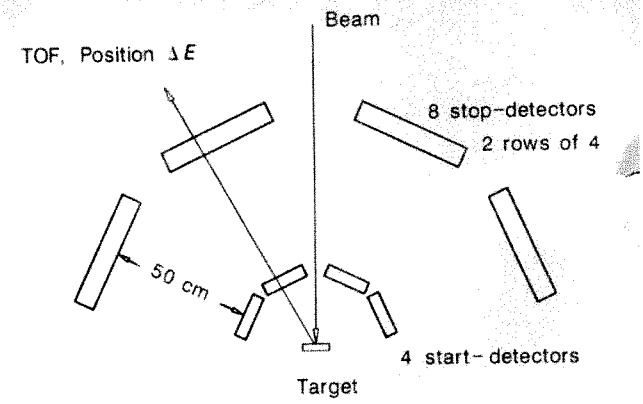


Fig. 1 Schematic diagram of the detection system. Four modules of multi-wire proportional counters are installed symmetrically around the beam axis (top view). Each detector module consists of a single start- and two stop-detectors, placed one above the other.

quantities. The time-of-flight (TOF), or equivalently the velocity of each scattered nucleus is calculated from the time signals of start- and stop-detectors. The specific ionization (ΔE), which depends on the atomic number Z and the velocity v of the nuclei according to $\Delta E \propto (Z/v)^2 \ln(v^2)$, is measured by the stop-detectors, and, by calibrating ΔE against TOF, ΔE therefore characterizes the atomic number of each nucleus (Fig. 2). The trajectory and, in turn, the scattering angle is obtained from the position measurements in the start- and stop-detectors.

A target of ~1–2 mm thickness, prepared from an iron meteorite (Sam's Valley meteorite, Medford, Oregon, 1894), was irradiated with 1.7×10^{14} particles of ^{238}U. Figure 2*a* and *b* shows the two-dimensional spectra, in terms of ΔE as a function of TOF, of particles in the angular regions 92°–120° and 127°–170°, respectively. No data were recorded below $\Delta E \approx 110$ (in relative units) because of an electronic cutoff.

The solid curves in Fig. 2*a*, *b* represent the 2σ range (95.4% probability) of ΔE-TOF values for ^{238}U scattered from a thin uranium target in a calibration experiment. No scattered uranium nuclei were recorded for a TOF below 90 ns. Some uranium nuclei with a TOF greater than 90 ns are seen at 92°–120° (Fig. 2*a*), but no uranium nuclei were recorded at angles exceeding 127° (Fig. 2*b*). This strong decrease in the number of scattered nuclei with angle is typical of multiple scattering, and for this reason the events with TOF > 90 ns were excluded from the analysis. The absence of events between TOF ≈ 30 ns, which corresponds to the initial velocity of uranium nuclei, and 90 ns can be used to estimate limits for the abundance of supermassive nuclei in a certain mass range. The lower limit of this mass range is determined by TOF = 90 ns, which corresponds to backscattering of uranium from nuclei with mass $A \approx 400$ located at the target surface.

A group of scattered nuclei with TOF centred around 70–80 ns, with specific ionization much lower than that for uranium ions of the same velocity, was observed at angles of 92°–120° (Fig. 2*a*). From comparison with the calibration curves, this group was identified as comprising nuclei with $Z \approx 30$, which we thus attribute to iron nuclei. The dashed curve in Fig. 2 shows the loci of iron nuclei as calculated from tabulated energy loss values[7]. As iron is the main component of the meteorite, an observation of scattered iron nuclei is not surprising. Such events can result from rescattering of iron recoil nuclei produced in primary uranium–iron collisions. We emphasize here that the identification of nuclei does not require any knowledge of the underlying scattering process (single or multiple), but is based entirely on the experimental calibration curves of ΔE against TOF.

To calculate upper limits for the abundance of supermassive nuclei with mass A, the Rutherford-scattering cross-section an

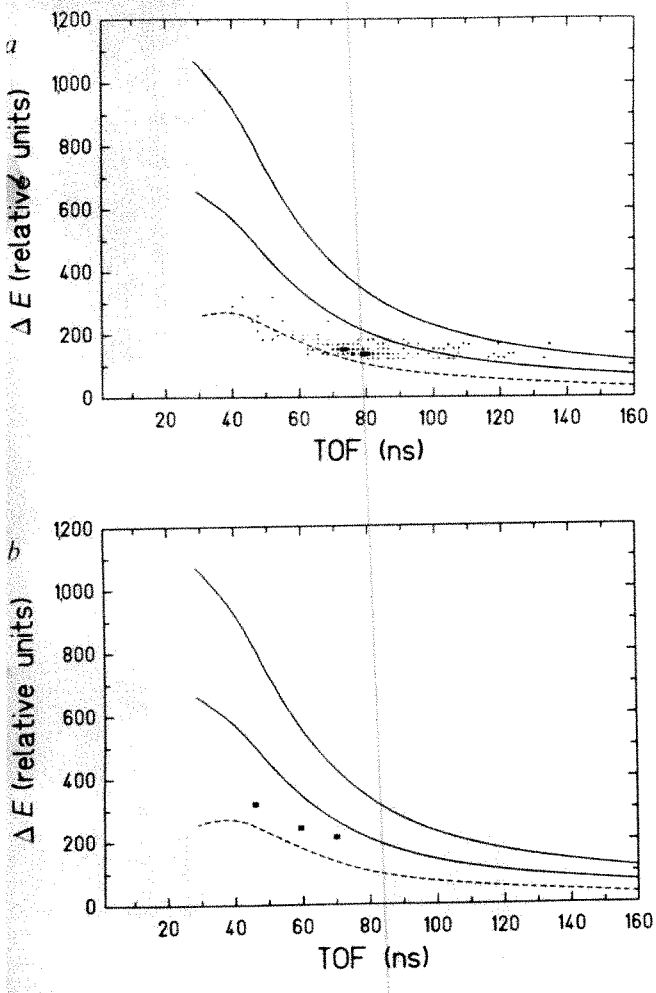


Fig. 2 Two-dimensional spectrum of specific ionization (ΔE) against time-of-flight (TOF) in the angular regions 92°–120° (*a*) and 127°–170° (*b*). The points represent the loci of a total of 245 (*a*) and 3 (*b*) detected particles. No data were recorded below $\Delta E \approx 110$. The initial projectile velocity corresponds to a TOF of 30 ns. The dashed line indicates the calculated locus of ^{56}Fe nuclei and solid lines span the 2σ range for scattered ^{238}U from a calibration experiment.

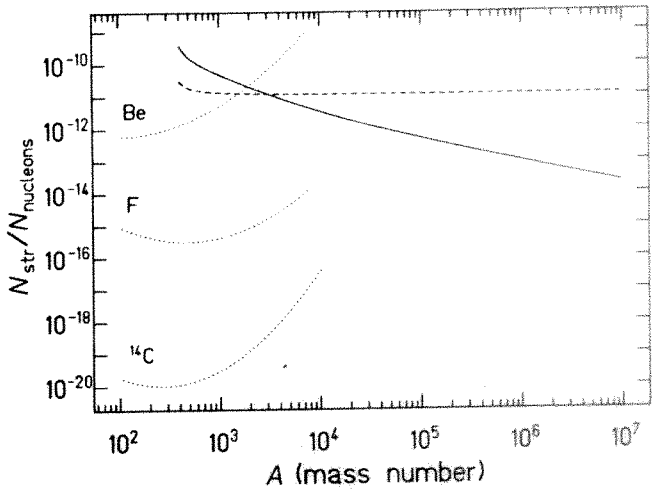


Fig. 3 Upper limits for the abundance of strange nuclei and supermassive relic particles (N_{str}) relative to the number of nucleons ($N_{nucleons}$) as a function of the respective mass A. The solid line represents the upper limit for the abundance of strange nuclei from Rutherford backscattering of ^{238}U in an iron meteorite, and the dashed line shows the upper limit for the abundance of supermassive relic particles from Rutherford backscattering of ^{238}U in a lanthanide sample. Dotted lines show upper limits for the abundance of supermassive relic particles from a search for heavy isotopes of light chemical elements[9].

the effective target thickness must be known. The latter depends on the scattering angle and the mass A. The charge Z, as a function of A, of the supermassive nucleus determining the Rutherford cross-section was taken from the calculation in ref. 2 (with parameter values $E/A = 899$ MeV and $\alpha_c = 0.6$). With other values of these parameters, the upper limits decrease by up to a factor of 20, so that our estimate is model dependent. The Rutherford cross-section was calculated assuming point-like colliding nuclei, which is a good approximation for masses of (strange) nuclei up to 10^4 because the minimal distance between the nuclei is several times their size. For larger masses the minimal distance from the surface of the strange nucleus to the projectile becomes comparable with the size of the strange nucleus, which could necessitate corrections to the Rutherford formula. At present, our knowledge of strange nuclei is insufficient to enable such corrections to be calculated. The upper limits are shown by the solid line in Fig. 3 as a function of the mass number A of the supermassive nucleus. There is increasing sensitivity with increasing mass because of the corresponding increase in the Rutherford cross-section.

The screening of the nuclear charge by electrons inside a large strange nugget could be important, making an estimate of the effective charge difficult. As Farhi and Jaffe explain[8], no appreciable screening occurs up to $A \approx 10^7$. We did not extend the estimated limits beyond $A \geq 10^7$ because above this, no reliable information on the screening was available. It is also important to note that the Coulomb barriers of strange nuggets are high enough[8] (except for nuggets of low atomic number) that elastic scattering is dominant at energies of 1.4 MeV per nucleon.

In addition to the iron meteorite, several terrestrial samples were studied using ^{238}U and ^{208}Pb beams: garnets, manganese nodules and the group of lanthanide elements extracted from the minerals apatite, monazite and bastnesite. The combined upper limits, not shown, are two times lower than those for the iron meteorite.

The dotted lines in Fig. 3 show results of a search for supermassive isotopes of light chemical elements, using an electrostatic spectrometer[9]. These isotopes were expected to be ordinary nuclei containing supermassive particles that are relics of the Big Bang[10–12]. The upper limits obtained for the abundance of supermassive isotopes with masses between 10^2 and 10^4 were rather low; we can consider these limits to be those for strange matter as well.

Our results, on the other hand, can be interpreted as giving upper limits for the abundance of supermassive relic particles that are bound to nuclei by hadronic interactions so that they cannot be detached in a collision process. Such limits are shown by the dashed line in Fig. 3 for relic particles attached to lanthanide elements. These limits can be considered as complementary to the previously published results (ref. 9 and references therein) because they cover a range of masses ($>10^4$ AMU) which was not investigated in ref. 9.

From our present knowledge of strange matter[2] we do not expect its properties and abundance to fluctuate from one atomic number to the next. Thus, a further improvement in sensitivity can be achieved with chemically enriched targets, particularly by using chemical separations[12] of elements which do not occur naturally, such as technetium[13], plutonium[14] or promethium. Strange matter with atomic numbers of these elements should be as stable and as abundant as that of neighbouring atomic numbers. Thus, by chemically separating these elements from bulk material it is possible to obtain targets enriched in strange matter by several orders of magnitude. A similar reasoning applies to supermassive relic particles[12].

Our limits for the abundance of strange matter are close to the instructive estimates of De Rújula and Glashow[3]. At present we do not consider it useful to put constraints on these theoretical considerations, because, for the samples investigated here, we cannot exclude the possibility of appreciable fluctuations in the concentration of supermassive nuclei caused by fractionations during chemical processes in the early Universe. Further studies of primitive meteorites, which have not been subjected to gravitational effects and whose composition reflects the primordial abundance of nuclei, may provide appropriate data from which to extract such constraints.

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Growing drops of strange matter

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Strange matter comprises roughly equal numbers of up (u), down (d) and strange (s) quarks, in contrast to nuclear matter, for which s quarks are absent. The theoretical possibility[1–7] that S-drops (small 'bags' of strange matter) may not only be metastable, but might become absolutely stable if grown to sufficient size, would have consequences of the greatest importance. In particular, this form of matter could provide the basis for a compact energy source. Here we describe a hypothetical experiment to explore this possibility. The first step is the production and detection of S-drops in relativistic heavy-ion collisions. Previously, we have estimated[8] the efficiency of production of S-drops by the process of fragmentation of quarks into hadrons following formation of hot quark–gluon matter. The proposed experiment would initially produce and detect S-drops of charge $Z=-1$ in present CERN and BNL facilities. The next step would be to grow small, positively charged S-drops to large stable S-drops through neutron capture in a confining apparatus. These two steps could provide the scientific basis for subsequent engineering studies of S-drops as an energy source.

Considerable theoretical interest has centred on the intriguing possibility that not only may S-drops be very long-lived, but large extended such objects, comprising strange matter (S-matter), might be absolutely stable. A nucleus of baryon number A consists of neutrons and protons, each of which is made up of three (massless) non-strange quarks (udd and uud respectively) confined to a small 'bag'. Consider a single bag enclosing a total of $3A$ u and d quarks. This has a higher energy then the standard nucleus A. By replacing some u and d quarks by s quarks, however, the Fermi energies in the system can be lowered significantly. Neglecting the strange quark mass, m_s, the number of strange quarks n_s in this S-drop should be

$$n_s = n_u = n_d = A$$

Chin and Kerman[3] calculated that S-drops with $A > 10$ and $n_s/A > 0.8$ would be metastable with lifetimes $\tau > 10^{-4}$ s. More recently, Witten[5] made the startling suggestion that very large S-drops, indeed S-matter, with $n_s/A \approx 1$ might be absolutely stable, that is, the energy per baryon is <938 MeV. This has important implications for S-drops: neutron stars might actually consist of S-matter with essentially no crust, a possibility with interesting astrophysical consequences[5,9–13]. Of more general significance, however, is the possibility that a drop of stable S-matter could be used as an energy source, because, unlike nuclei, S-drops become more stable as they grow. It is this exciting idea that we wish to discuss here.

Using the Fermi-gas model with lowest-order quantum chromodynamics (QCD) corrections, Farhi and Jaffe[6] calculated that S-matter could be stable for an appreciable range of values of the QCD coupling strength and of m_s. Bethe *et al.*[14] argued against S-matter having a lower energy than nuclear matter. However, those authors approximated S-matter as being equivalent to an interacting gas of Λ particles, which is not a convincing model. As it seems likely that QCD calculations will not be accurate enough in the foreseeable future to give a definitive prediction on the stability of S-matter, experimental

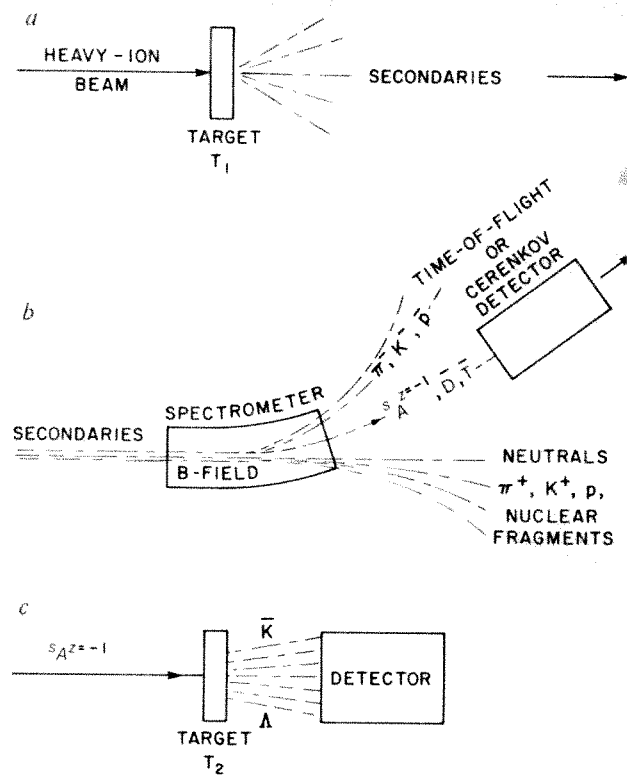


Fig. 1 A schematic diagram of the proposed high-sensitivity experiment to detect $Z=-1$ S-drops at CERN and BNL. *a*, Literally hundreds of secondaries will be produced in several types of processes on collision of the beam with target T_1. *b*, S-drops $^{S}A^{Z=-1}$ with momentum given by equation (1) are separated in the magnetic spectrometer. *c*, Positive identification of separated relativistic S-drops is made by secondary collisions in the downstream target T_2, producing a large number of $\bar{K}$ and Λ particles.

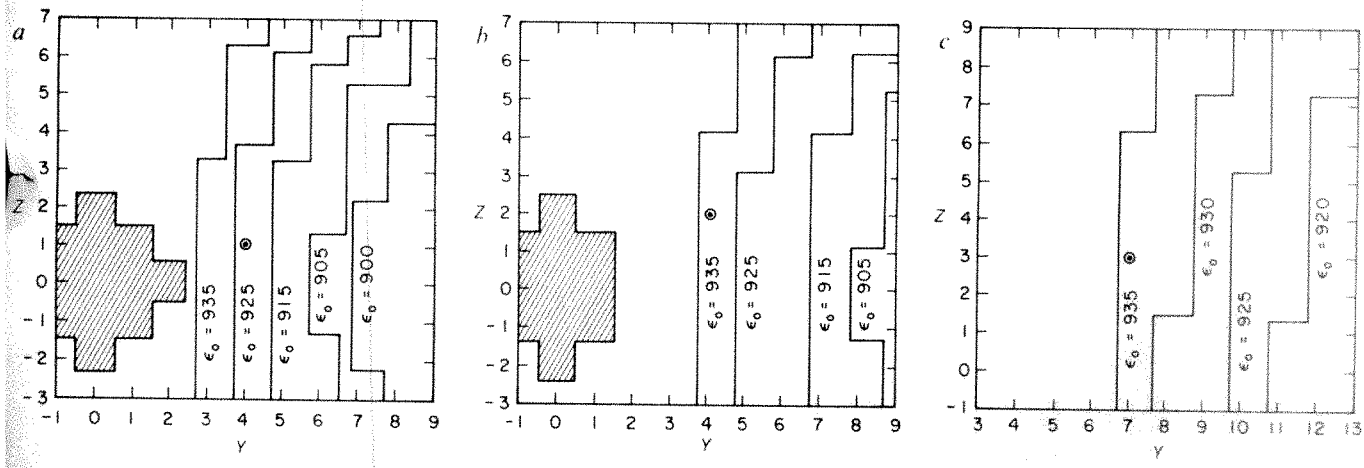


Fig. 2 Stability of S-drops against neutron emission by the strong and weak interactions for $m_s = 150\ \mathrm{MeV}/c^2$, calculated using the mass relations of Berger and Jaffe[7] for ${}^{S}A^{Z}$ as a function of ε_0, the energy per baryon for stable infinite S-matter. *a*, For $A = 15$, the S-drop is stable against neutron emission ($\Delta Y = -1$, $\Delta Z = 0$, $\Delta A = -1$) by the strong interaction if (Y, Z) is in the region to the left of the solid line for a given ε_0. Here, $Y = A + S$ is the hypercharge. For $\varepsilon_0 \leq 920$ MeV, the S-drop is also stable against neutron emission ($\Delta Y = 0$, $\Delta Z = 0$, $\Delta A = -1$) through the weak interaction for all (Y, Z). The unstable region against weak neutron emission, for $\varepsilon_0 = 925$ MeV, is shown by the shaded region. The solid circle at $(Y, Z) = (4, 1)$ is the location of the lowest-energy $A = 15$ S-drop. This is an S-drop ${}^{S}A^{Z} = {}^{-11}15^{1}$ with $n_d = 18$, $n_u = 16$ and $n_s = 11$. *b*, Same as *a*, but for $A = 20$. Again the shaded region corresponds to $\varepsilon_0 = 925$ MeV, and is a smaller domain than that in *a*. $(Y, Z) = (4, 2)$ is the energy minimum. *c*, Same as *a*, but for $A = 40$. For $\varepsilon_0 = 925$ MeV, there is no region unstable against weak neutron emission.

tests are necessary and warrant prompt attention in view of the significance of a positive outcome.

Consider step 1 of our proposed scenario, the production and detection of S-drops in the present relativistic heavy-ion-beam (fixed-target) facilities at CERN (200 GeV per baryon) and BNL (14.5 GeV per baryon). We assume that, as in the 'standard' picture of such collisions, a high-density, hot quark–gluon (QG) drop is formed[15]. We stress that the probability for forming such a QG drop need not be large and that a phase transition to a QG plasma need not occur for our proposal to be viable. (No real evidence for the formation of QG plasma has been found in the recent experiments[16] at BNL and CERN.) Liu and Shaw[8] calculated the emission of mesons and baryons in several (strong) interaction processes from a QG-drop precursor of an S-drop. The dominant process was the emission of high-momentum u and d quarks which 'fragment' and pick up an $\bar{s}$ quark from an $s\bar{s}$ pair to form a meson, leaving behind an s quark. (See also the mechanism for S-drop production of Greiner *et al.*[17].) The formation of many strange particles is typical of the observed events at CERN. Although model-dependent, the possibility of producing an S-drop with $n_s/A > 0.8$ from the QG drop was much greater for small-A ($\lesssim 30$) drops, this probability being quite appreciable. We imagine that an S-drop cools to a metastable state ${}^{S}A^{Z}$ (each s quark contributing a strangeness $S = -1$) mainly by the emission of mesons and photons. A range of states ${}^{S}A^{Z}$ will be produced. Although the detection of these S-drops is conceptually simple (because of the low Z/A ratio), it is technically very difficult because of the huge multiplicity of particles produced in a head-on collision.

We propose that a search be made for $Z = -1$ S-drops at CERN and BNL. Consider an S-drop ${}^{S}A^{Z=-1}$ produced in the QG matter formed from the relativistic collision between a beam heavy-ion with energy γ GeV per baryon ($\gamma \gg 1$) and a fixed-target heavy nucleis (Fig. 1*a*). We assume that on average each nucleon in a beam-ion interacts with one nucleon in the target nucleus; this defines a rest frame for S-drop production. The laboratory momentum of the S-drop then is

$$P_S \approx c(\gamma/2)^{1/2}({}^{S}A^{Z=-1})\ \mathrm{GeV}/c \qquad (1)$$

in a nearly forward direction. The key to the separation of these S-drops from other products in the magnetic spectrometer in Fig. 1*b* is that if A is greater than some reasonable minimum (~ 15) the S-drops will have a larger momentum than all the relevant background secondaries, specifically negatively charged π^-, K^- and $\bar{p}$. These latter particles, with mass m, would have momentum $P \lesssim c\gamma m$. In a field B, the spectrometer will bend particles in an arc of radius R according to $(P/Z)\ (\mathrm{GeV}/c) = 0.3\,BR$ (with B in tesla and R in metres), so that, taking $m = 1\ \mathrm{GeV}/c^2$, for ${}^{S}A^{Z=-1}_{\min} > \sqrt{2\gamma}$, all negatively charged π^-, K^- and $\bar{p}$ will be bent more than the S-drops, which may thus be separated from these negatively charged particles (as well as from the neutral and positively charged secondaries). At CERN, heavy-ion beams[16] having $\gamma = 60$ and 200 have been used, so that ${}^{S}A^{Z=-1}_{\min}$ will be 11 and 20 respectively. The only 'standard' background would come from $Z = -1$ antideuterons ($\bar{D}$) and antitritons ($\bar{T}$) which are produced in proton-nucleus collisions with rates[18] $\bar{D}/\pi^- \lesssim 10^{-5}$ and $\bar{T}/\pi^- \lesssim 10^{-9}$. The BNL heavy-ion beam[16] has $\gamma = 14.5$, and thus will not produce a $\bar{D}$ or $\bar{T}$ background. Central collisions might also produce negative, fractionally charged free quarks as a background; limits of $< 1.0 \times 10^{-9}$ quarks per incident oxygen ion have been set at BNL[19]. Following magnetic separation, Čerenkov detectors or time-of-flight detectors can then be used to distinguish between the ${}^{S}A^{Z=-1}$ drops and the low background of $\bar{D}$ or $\bar{T}$ at CERN. Finally, positive identification of these separated relativistic S-drops could be made in secondary collisions in the downstream target (Fig. 1*c*). The centre-of-mass energies will produce excitations much greater than the total binding energy of the S-drop, which will then disintegrate into a large number of $\bar{K}$ and Λ particles, giving a readily detectable and distinctive signature.

For an S-drop of given A, it is expected that the minimum-energy state will have positive Z. As can be seen from the mass relations of Berger and Jaffe[7], S-drops with negative Z will not be stable. (Perhaps this is fortunate, as stable negatively charged S-drops might interact with normal matter to grow without bounds.) However, we see that, for a considerable range in parameters, the $Z = -1$ S-drops will live sufficiently long (Figs 2 and 3) to perform the high-sensitivity experiment depicted in Fig. 1. More quantitative details will be published elsewhere.

Assuming that the production of these small $Z = -1$ S-drops is indeed detected as outlined above, let us turn to step 2, the growing of S-drops for their use as an energy source. It is clear that an intermediate step would be to optimize the production

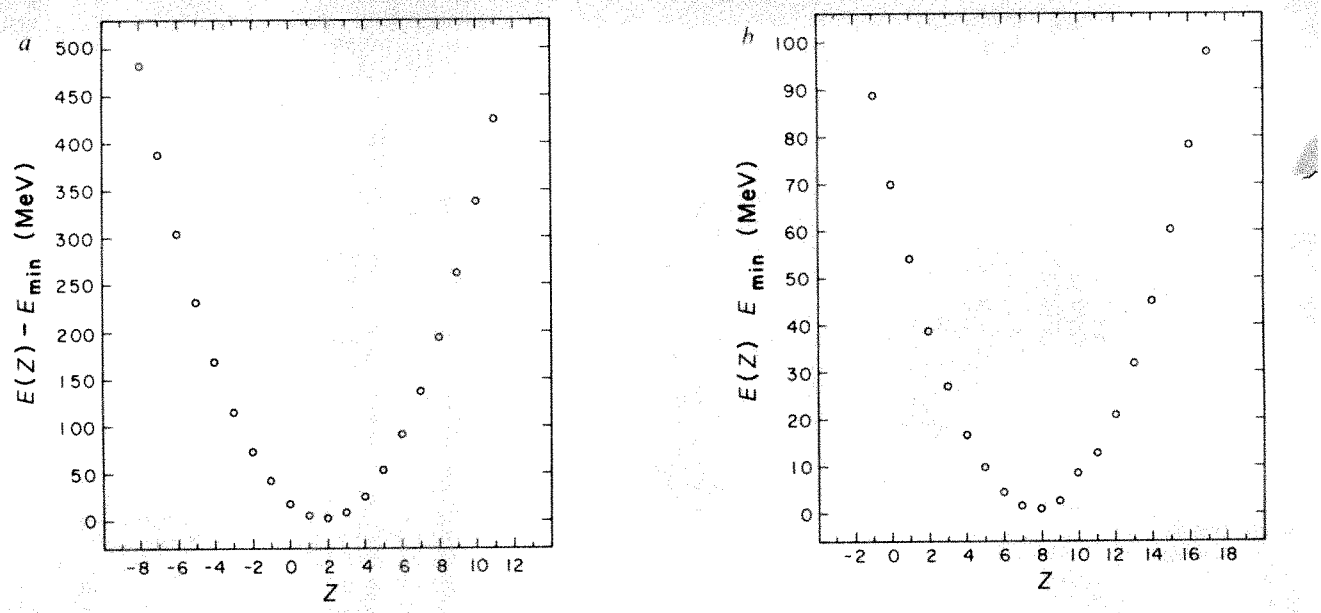


Fig. 3 *a*, Energy of the S-drop $E(Z)$, calculated using the mass relation of ref. 7, as a function of Z relative to its minimum value E_{min} ($\equiv E(Y = Y_{min} = 4, Z = Z_{min} = 2)$), for $A = 20$, $Y = Y_{min}$, $\varepsilon_0 = 920$ Mev and $m_s = 150$ MeV/c^2. This relative energy is rather insensitive to the choice of ε_0 (in the range 900–935 MeV). We note that an S-drop ${}^{S}A^{Z} = {}^{-16}20^{-1}$ would have an energy $\Delta E \approx 40$ MeV above the ground state ${}^{-16}20^{2}$, and $\Delta E \approx 20$ MeV above the ${}^{-16}20^{0}$ state to which it will decay by β-emission ${}^{-16}20^{0} + e^{-} - \bar{\nu}_e$. By scaling tabulated β-decay rates, we obtain the lifetime $\Gamma_{\Delta Z=1,\Delta S=0} \approx 10^{3}\,(\Delta E\ (\mathrm{MeV})/20)^{5}\ \mathrm{s}^{-1}$. Thus, this $Z = -1$ S-drop would live much longer than is necessary to perform the experiment proposed in Fig. 1. *b*, Same as *a*, but for $A = 100$ (for which $Y_{min} = 19$ and $Z_{min} = 8$).

and separation of the metastable (positive-Z) S-drops. We note that experimental and theoretical examination of the S-drops so produced, and their decays, will provide important information concerning the nature of the mass formula. The energy per baryon of stable infinite S-matter, ε_0, will thus be determined, as well as the quantitative details of the shell effects considered by Takahashi and Boyd[20].

The S drops produced and separated in the heavy-ion collisions should be grown as quickly as possible for at least the following reasons: (1) although stable against neutron emission for certain values of ε_0 (see Fig. 2), these S-drops might be unstable against α-decay, with lifetimes which, because of the Coulomb barrier, are long on experimental timescales (seconds) but not in relation to the needs for storage; (2) their binding energy per baryon grows with A; (3) their neutron-absorption cross-section increases as $A^{2/3}$; (4) their 'sticking' probability to a surface increases with A (that is, their thermal velocity decreases). Thus we propose a technique for rapid growth of relatively small (perhaps $A \geq 30$) S-drops to considerably larger (perhaps $A > 10^4$) ones, by passing through liquid deuterium in a confining apparatus as shown in Fig. 4. The small relativistic metastable ${}^{S}A^{Z=-1}$ drops are slowed down in a linear electrostatic decelerator to non-relativistic velocities. Near the end of this decelerator would be a tank of liquid hydrogen of length such that an S-drop passing through it would pick up two protons and emerge with a charge $Z = 1$. (Once $Z = -1$ S-drops are detected, one could infer that $Z > 0$ S-drops are also being produced at still higher rates. A major effort should then be made to optimize the production and the separation of these $Z > 0$ S-drops. This in turn would enable $Z > 0$ S-drops to be injected directly into the decelerator, obviating the need for a liquid-hydrogen tank.) An emerging ${}^{S}A^{Z=1}$ drop is then circulated through the liquid-deuterium tanks, where it picks up neutrons with a cross-section that we estimate (from nuclear (D, p) reactions) to be $\sim(A/30)^{2/3}\ 10^{-25}\ \mathrm{cm}^2$. An absorbed neutron in the S-drop would project onto its quark degrees of freedom and decay very rapidly by γ-transitions to the lowest allowed u and d levels. As neutrons are absorbed, the Fermi level of the d quarks increases relative to that of the s quarks, until a d → s transition takes place with subsequent γ-emission: following neutron capture, an S-drop ${}^{S}A^{Z}$ will relax towards it energy minimum by strangeness-changing weak decay ${}^{S}A^{Z} \rightarrow ({}^{S-1}A^{Z})^{*}$, which essentially changes a d quark to an s quark (u + d → s + u) in a charged weak-current interaction. The asterisk indicates an excited state, which decays rapidly by γ-emission. Scaling the similar decay ($K^{+} \rightarrow \pi^{+}\pi^{0}$), we obtain a decay rate

$$\Gamma_{\Delta S=-1,\Delta Z=0} \approx 10^{5}\left(\frac{\Delta E(\mathrm{MeV})}{20}\right)^{2}\ \mathrm{s}^{-1} \qquad (2$$

The $E(Y)$ curves required to evaluate (2) (where Y is the hyperchange $A + S$) are numerically very similar to the $E(Z)$ curves in Fig. 3.

Thus the S-drop grows and becomes more stable; we envisage small initial S-drops ($A \approx 30$–50, for which $Z_{min} \approx 3$) growing to a size $A \geq 10^4$. Our rough calculations of absorption length, Coulomb barrier, time constants and so on seem reasonable for

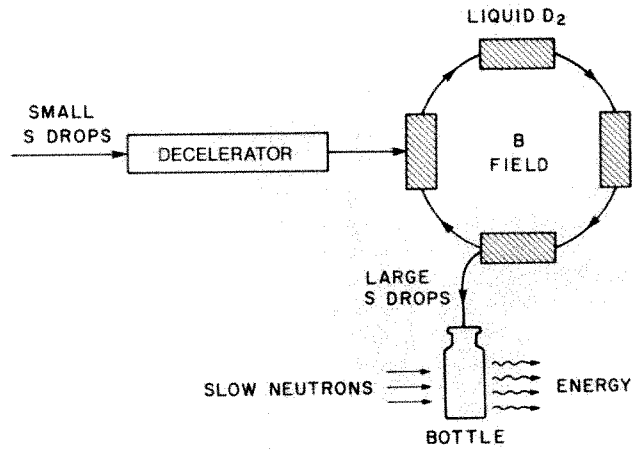


Fig. 4 Schematic diagram of proposed apparatus for the rapid growing of small S-drops into large S-drops. These can be stored and used as a compact energy source, by feeding in slow neutrons. For each neutron absorbed by an S-drop, $\sim(938 - \varepsilon_0)$ MeV of energy is released (mostly in γ-rays).

ne values $B \approx 1$ tesla, S-drop velocity $v/c \approx 0.1$ and a radius or the ring of tanks of ~5 m. Some acceleration of the S-drop etween liquid-deuterium tanks would be necessary to maintain $/c \approx 0.1$. The large-A S-drop may then be put into a 'conven-ional' storage bottle, where it decays to its stable Z_{min} and Y_{min} or that A. (Note that if we start from small Z values, and keep lways below Z_{min}, the Coulomb barrier for the S-drop will nhibit the possibility of α-decay until it has grown sufficiently arge to be stable against such decay.) These large stable S-drops ould then be used as a compact energy source.

Because stable S-matter may exist for a range of QCD par-meters, one might hope to be able to investigate its formation xperimentally. Our proposal, outlined schematically in Fig. 1, or producing and detecting small ${}^{S}A^{Z=-1}$ drops could be carried ut readily in present CERN and BNL relativistic heavy-ion ixed-target facilities. Our next proposal, outlined in Fig. 4, for apid growth of these small S-drops into larger ones, to be stored nd used subsequently as an energy source, seems plausible. We emphasize that our calculations of energies and lifetimes, given in Figs 2 and 3 and based on mass relations[7] for ${}^{S}A^{Z}$, hould be considered as only illustrative. No fine tuning of arameters is necessary for our general scheme to be viable. The use of the stable S-drops stored in the bottle (Fig. 4) as an nergy source appears conceptually simple. Slow neutrons are fed into the bottle; for each neutron absorbed by a large S-drop, an energy of $\sim(938 - \varepsilon_0)$ MeV is released, almost entirely in the form of γ-rays, and the S-drop becomes larger and more stable. In conclusion, therefore, we suggest that if S-matter is stable the production and growth of stable S-drops should be feasible and of considerable theoretical and pragmatic interest.

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Late-time optical spectra of SN1987A

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Optical study of late-time nebular emission provided the first direct evidence for the presence of radioactive ^{56}Co in the ejecta of Type Ia supernovae[1] and yielded conclusive evidence that Type Ib events constitute a physically distinct class of supernovae[2]. Hydrogen-rich Type II supernovae have not been studied in detail in the nebular phase, but SN1987A provides an unprecedented opportunity to probe the structure and composition of the ejecta by modelling the nebular spectrum. Here we investigate the late-time nebular spectra of SN1987A by computing the emergent spectra from model atmospheres. The new features of this calculation are the inclusion of a complex model hydrogen atom and the self-consistent treatment of an opaque lower boundary which approximates the observed continuum. We adopt hydrodynamic models reported in the literature, which are known to reproduce the observed light curve and γ-ray and X-ray spectra, and we obtain qualitative agreement with observed optical spectra. By examining the effect of mixing core material into the envelope, we conclude that the metals in the hydrogen-rich envelope make a substantial contribution to the optical nebular lines even for models with no outward mixing of the core material; however, a significant fraction of the energy emitted in ^{56}Co decay must be deposited in the envelope to produce the observed linewidths and hydrogen line strengths.

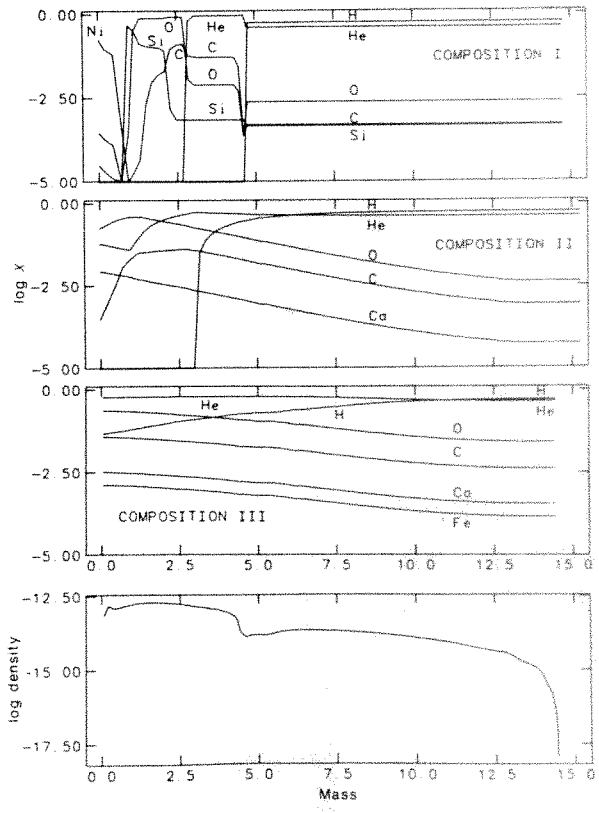


Fig. 1 Mass fraction X for several representative elements plotted logarithmically against mass for three mixed-composition models. Composition I (top) results from a standard one-dimensional hydrodynamic calculation. Compositions II (upper middle) and III (lower middle) are *ad hoc* mixed versions of composition I. The models correspond to models 10H (ref. 5), 10HM (ref. 9) and 10HMM (ref. 11), provided by S. Woosley (private communication). In the bottom panel is plotted the logarithm of the mass density against mass. Note the steep gradient near 5 $M_\odot$, corresponding to the helium/hydrogen interface of the initial model, which indicates a natural delineation between 'core' and 'envelope'. The neutron-star contribution has been removed from these plots and the mass coordinate rescaled appropriately.

Synthetic spectral diagnostics have been applied to the late-time conditions in supernova 1987A by Fransson and Chevalier[3]. They assumed a stratified core composition, with the radioactive heating source confined to the central regions, and neglected the hydrogen envelope. Their calculations did not incorporate the possibility of mixing of the composition. Recent theoretical investigations conclude that a large amount of mixing of the composition, and particularly of the radioactive ^{56}Co heating source, is required to accurately reproduce the light curve[4-10] and the γ-ray and X-ray spectral development[11-16].

Here we analyse the mixing scenario by presenting optical spectra from models with different degrees of mixing. We distinguish between 'composition mixing', which implies a smoothing of some part of the stratification that arises naturally in one-

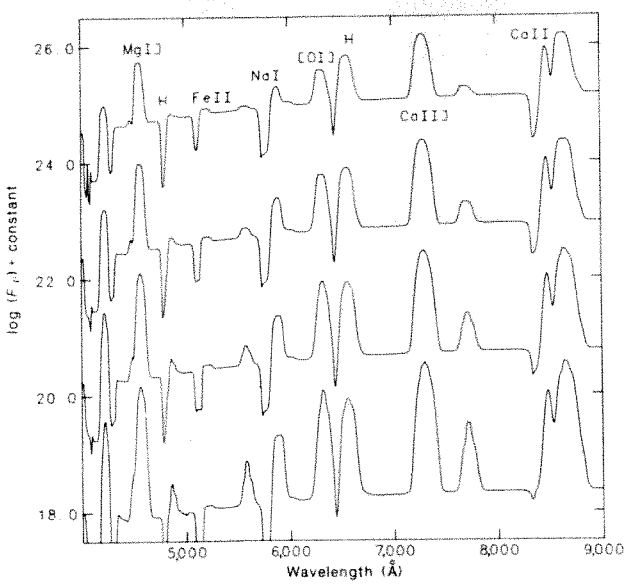


Fig. 2 Spectra for composition III at 200 days for various photospheric radii. From bottom to top, the radii are $R_{ph} = 1.5$, 2.0, 3.0 and 4.5×10^{15} cm, corresponding to mass points 1.1, 3.0, 4.6 and 7.5 $M_\odot$. For clarity the spectra have been shifted relative to each other by two logarithmic units on the vertical axis. Note the contribution to the lines from the 'envelope' mass (upper spectrum). In the lower two spectra, spikes are evident at the centre of the line profiles, arising from contributions from the exposed, slow-moving core material.

dimensional hydrodynamic models, and '^{56}Co mixing', which can be imposed on the model while maintaining stratification of the remaining elements. The distinction between composition mixing and ^{56}Co mixing is necessary to clarify our conclusions. One might imagine that the mixing of ^{56}Co, without mixing of the other elements, appears contrived. The possibility exists, however, that freshly synthesized material created in the deepest layers could penetrate into the envelope in the form of small, energetic 'clumps', which could provide extensive ^{56}Co mixing while preserving the original stratification of the remaining composition.

The techniques used here for computing the late-time spectra are similar to those employed previously for supernovae[1,17,18] and for SN1987A in particular[3]. The equilibrium structures of temperature and of ionization are found by imposing radiative and thermal equilibrium. Radiative equilibrium requires that heating of the gas must be balanced by radiation escaping the gas. It follows immediately that any given model will underestimate the total number of emission lines present in the real atmosphere and thus that the model can provide only an upper limit to the emissivity of the lines that are included.

We solve the radiative-transfer equations for the lines using the Sobolev approximation[19,20] with modifications[21,22] applicable to many-level and many-ion conditions. Special modifications necessary for solving the combined line-transfer and statistical-equilibrium equations for hydrogen will be discussed elsewhere (D.A.S., manuscript in preparation). We ignore continuum transfer. We do, however, mimic the effect of an optically thick inner region by imposing a lower boundary condition with a prescribed intensity I_ν^{inc} incident from below. The radius, R_{ph}, of this 'photosphere' and the frequency variation of the continuum are free parameters in the models. We require that the total luminosity L from the opaque region equals the true luminosity of the material that is heated radioactively below R_{ph}. We choose the continuum shape to be represented by a diluted black body at temperature T_{BB}, and define $L_{BB} = 4\pi R_{ph}^2 \sigma T_{BB}^4$. The dilution factor $\delta \equiv L/L_{BB}$ is fixed by energy conservation. The incident intensity is thus $I_\nu^{inc} = \delta B_\nu(T_{BB})$. For all models reported here a rough fit to the observed continuum shape is achieved with $T_{BB} = 5{,}000$ K. The dilution factor depends both on the selection of R_{ph} and on the extent of ^{56}Co mixing. The latter determines the actual luminosity at the photosphere. Many weak lines combine to form a quasi-continuum in the observer's frame which is smoothed further by electron scattering. Electron scattering will both broaden the observed line profile and decrease the luminosity at the line centre, because of the differential expansion of the atmosphere[18]. Our photosphere effectively smooths the detailed line spectrum that would be emitted from the material below R_{ph}, replacing it with a continuous spectrum. The core material of the atmosphere has a higher density and metal content (metallicity) relative to the envelope (see below); therefore, the electron density is relatively large and many weak metal lines are formed in the core. Our lower boundary condition most reasonably represents the natural conditions when it is placed within or immediately above the core.

This study is confined to hydrodynamic models and composition-mixing schemes for supernova 1987A that have been presented already in the literature. The canonical model comprises a 6-$M_\odot$ core, composed chiefly of helium and oxygen, and a 10-$M_\odot$ hydrogen-rich envelope. The inner 1.4 $M_\odot$ forms a neutron star. Three composition-mixing variations for this model are shown in Fig. 1. The variations correspond to models 10H (ref. 5) (composition I), 10HM (ref. 9) (composition II) and 10HMM (ref. 11) (composition III), which were provided by S. Woosley (private communication). The increased composition mixing represents successive improvements to the theoretical light curves and γ- and X-ray spectra reported in refs 5, 9 and 11.

We present results that are valid at 200 days after the supernova event, when the deeper layers may be observable. The placement of the lower boundary, R_{ph}, and the distribution of ^{56}Co are the only free parameters of the models. The total mass of radioactive ^{56}Co in all models is 0.075 $M_\odot$. Theoretical spectra for the 'standard' Type II-supernova model, consisting of a stratified core composition and with the ^{56}Co confined to the central region, are not consistent with the observed spectra of SN1987A. We therefore concentrate on models that incorporate various degrees of mixing.

Spectra of several models computed using composition III with different photospheric positions are shown in Fig. 2. For these calculations, the radioactive cobalt is distributed with constant mass fraction out to a mass cut of 14 $M_\odot$. The best fit to the spectral line strengths and shapes corresponds to $R_{ph} = 3.0 \times 10^{15}$ cm, a radius just outside the 'core'. The best fit to the continuum flux also occurs for this value of R_{ph}, although this result depends to some extent on the ^{56}Co distribution as well (see below). This same value of R_{ph} also produces the best results for models using compositions I and II. The spikes seen in the lower two spectra of Fig. 2 are accentuated in those models that feature increased stratification. These spectral features arise from the increased density in the low-velocity core region and are stronger for higher metallicity. Most of the metal lines for models using compositions I and II are too strong if the core regions are left exposed. We conclude that broad-band opacity effects become important below the core/envelope boundary.

For each of the three compositions we have calculated spectra assuming that the cobalt is distributed below a mass cut M_c of 5, 10 and 14 $M_\odot$ and assuming that there is a constant mass fraction of ^{56}Co below the cut. These mass cuts correspond to maximum ^{56}Co velocities of 1.3, 2.5 and 3.8×10^3 km s^{-1} respectively. The resulting spectra for these ^{56}Co mixing variations, using composition III, are shown in Fig. 3, for which the value $R_{ph} = 3.0 \times 10^{15}$ cm is adopted. For $M_c < 10\ M_\odot$ the hydrogen lines are too weak and all the spectral lines appear too narrow, as can be seen dramatically in the Ca II infrared triplet. The

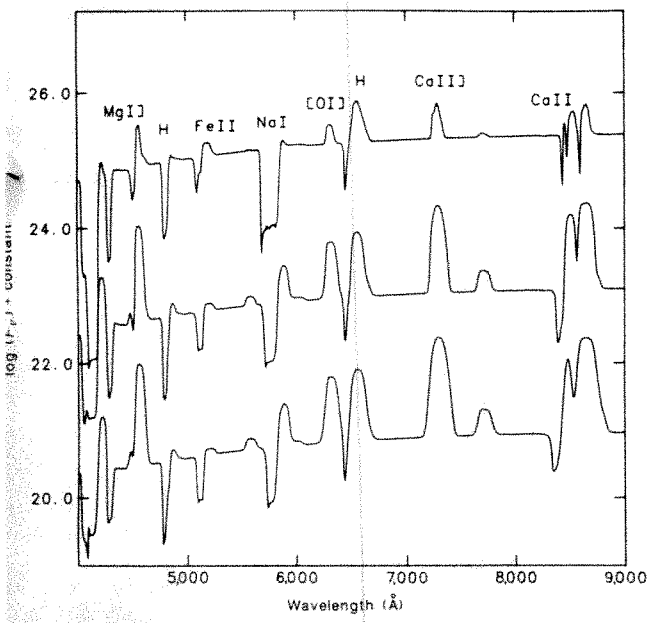


Fig. 3 Spectra for composition III at 200 days for different ^{56}Co mass distributions. The total ^{56}Co mass in all cases is 0.075 $M_\odot$. In all cases, the photosphere is located at radius $R_{ph} = 3.0 \times 10^{15}$ cm and the assumed temperature $T_{BB} = 5{,}000$ K. From bottom to top the ^{56}Co is distributed out to a mass point M_c of 14, 10 and 5 $M_\odot$. For clarity the spectra have been shifted relative to each other by two logarithmic units on the vertical axis. Note the increased continuum flux (most visible at the red edge of the spectra) for smaller M_c, which is due to the increased total luminosity from the opaque region. From bottom to top the luminosity at the photosphere is 5.7, 7.9 and 15.6×10^{40} erg s^{-1}.

same results occur for the other composition-mixing models with this value of M_c. The strength of the metal lines is satisfactory for composition III with $M_c = 5\ M_\odot$, because of the large mass of heavy elements mixed into the envelope in this model. The metal lines are not strong enough for compositions I and II when such a low value of M_c is used. We conclude that the best fit to the observed spectra occurs for $M_c \geq 10\ M_\odot$. Several studies[9-11] impose an outwardly decreasing mass fraction for the ^{56}Co distribution. We find that the optical spectrum is not sensitive enough to the details of the ^{56}Co distribution to differentiate between models with a gradient in ^{56}Co mass fraction and models with a constant mass fraction, provided that the decay energy is deposited throughout a major fraction of the ejecta. Our models require that the radioactive energy be deposited throughout a substantial fraction of the envelope; however, the cobalt could remain confined to the core if some mechanism exists for transporting the energy outward.

A comparison between the observed spectrum (M. Dopita, private communication) and the best results from the three mixing scenarios is presented in Fig. 4. The photospheric radius R_{ph} is 3.0×10^{15} cm (mass point 4.6 $M_\odot$) for all models, so that only envelope material contributes to the lines, and $M_c = 14\ M_\odot$. The lower metallicity of the unmixed model (composition I) provides the best overall fit, particularly at the red wavelengths, but all three models provide qualitative agreement with the observed spectrum. We cannot rule out any of these three models on the basis of these spectra, but composition I is preferred. The Ca II infrared triplet is too strong in all models by a factor of two or three. The Ca II] line strengthens as the metallicity increases and becomes too wide for composition III. As electron scattering tends to broaden the lines, we conclude that the fit for this line is poor for composition III. The strength of Hα is best for composition II although the red wing is weak in all models. The [O I] 6,300-Å line is accurately represented by the composition I model, as is the [O I] 6,300/[O I] 5,577 ratio. The Na I D-emission wing is too strong for all models by a factor

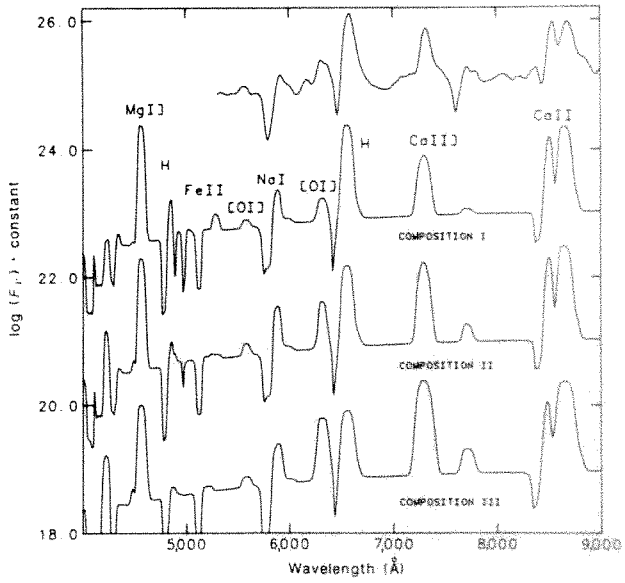


Fig. 4 Comparison of three mixed-composition models for observations at 200 days. From top to bottom the spectra are the observed spectrum (M. Dopita, personal communication) and models for compositions I, II and III. All models use $R_{ph} = 3.0 \times 10^{15}$ cm and $M_c = 14\ M_\odot$. For clarity the spectra are shifted vertically as before.

of two to three. At wavelengths shorter than those of the observed spectrum is the Mg I] 4,571-Å line. This line is very strong in all our models, including the many not discussed here. We note that this line is predicted to be strong by other models as well[3] but is not observed until much later times.

Based on these results, we conclude that (1) a good qualitative fit to the optical spectrum of SN1987A is obtained from the hydrodynamic models at 200 days if the lower boundary is placed just above the core; (2) the lack of observed narrow-line cores suggests that the dense slow-moving core region does not contribute substantially to the emission lines at 200 days; (3) a considerable contribution to the observed optical emission lines comes from the heavy elements in the envelope even if no composition mixing is invoked; (4) the radioactive decay energy must be distributed throughout a major fraction of the mass of the star ($M_c > 10\ M_\odot$) to be consistent with the observed spectrum; and (5) there is evidence for strong broad-band opacity at late times that is responsible for producing P-Cygni line profiles, as represented by our lower boundary.

Based on the limitations of computations used in this study, we further conclude that a large number of realistic model atoms, providing many spectral lines, is required to improve accuracy and that continuum-transfer effects should be included in future studies of this kind.

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Rapid variability of extragalactic radio sources

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Since its discovery more than 20 years ago[1,2], variability of extragalactic radio sources on timescales of weeks to years has been the subject of many investigations. Little is known, however, about radio variability of extragalactic sources on timescales of less than a few days. Variations on timescales of hours—mostly at millimetre wavelengths—have been reported for several sources[3–6]. Heeschen *et al.*[7] have found variability with amplitudes of up to 12% in less than 1 day at 11-cm wavelength in a sample of northern ($\delta \geq 60°$) compact sources. We have continued these observations at wavelengths of 6 and 11 cm using the 100-m telescope of the Max-Planck-Institut für Radioastronomie and report here the results for two sources. The quasar QSO0917+62 showed variations with amplitudes of up to 23% in ~24 hours, which were correlated at the two wavelengths; in the BL Lac object 0716+71 we found variations with amplitudes of 7–11%. We discuss intrinsic effects, gravitational lensing and scattering in the interstellar medium as possible explanations for rapid radio variability.

The observations were made in two sessions in 1988: 31 March to 5 April (112 hours, 6- and 11-cm observations made almost simultaneously) and 15–20 June (104 hours, 6 cm). For the 11-cm measurements we used a correlation receiver (cold-load reference) with a bandwidth of 80 MHz and a system temperature of 55 K; the 6-cm measurements were performed using a dual-beam correlation receiver with a bandwidth of 500 MHz and a system temperature of 65 K. Both receivers have circularly polarized feeds and are mounted in the secondary focus of the 100-m telescope. For each observing epoch, our sample included nine flat-spectrum compact sources (spectral index $\alpha_{11}^{6} \geq -0.5$; flux density $S \propto \nu^{\alpha}$) and seven or eight steep-spectrum reference sources of similar flux densities. The observations consisted of cross scans in azimuth and elevation through the source position, thus allowing for accurate correction of pointing errors (see Heeschen *et al.*[7] for details of the observations and data-reduction procedures, including corrections for gain and extinction). In addition, the influence of weak confusing sources was estimated from the difference of scans in azimuth and elevation as a function of parallactic angle; a corresponding correction was applied to the 11-cm data of six sources. The sum of all corrections applied to the raw data never exceeded 2%. For all sources we calculated a percentage fluctuation index $u = 100\ \sigma/\langle S \rangle$, where $\langle S \rangle$ is the mean flux density of the source in the observing session and σ is the standard deviation of single measurements. The errors are dominated by systematic errors (that is, proportional to S); their level is given by the mean fluctuation index for steep-spectrum sources, which is 0.27% at 11 cm and 0.35% at 6 cm wavelength. (The value at 11 cm wavelength reproduces exactly that of Heeschen *et al.*[7].) As none of the included steep-spectrum sources showed peak deviations of more than 1% from their mean flux densities (see for example 0951+69 in Fig. 1*a*), instrumental artefacts above this level can be excluded.

Of all observed sources, the quasar 0917+62 showed the largest variability during both sessions: 22% (peak-to-peak) at 11 cm and 14% at 6 cm in the March/April run, and 23% at 6 cm in June. In the figures, normalized variations $R = 100\ (S/\langle S \rangle - 1)$ are plotted against time. The fastest 'events' occurred at 6 cm wavelength in March/April; our ~75-min sampling after JD 2447255.4 reveals fine structure in the light curve on a scale of ~4 hours (Fig. 1*b*). The variations for the 11-cm data (Fig. 1*a*) are similar but much smoother; however, they show a remarkable discontinuity of the slope at JD 2447256.0, at about the same time as the secondary maximum at 6 cm. Two similar discontinuities are apparent in the 6-cm data for June (Fig. 1*c*), as well as a spike-like feature at JD 2447331.1, the amplitude of which is significantly above the uncertainty level. For the single observations of 0917+62 in March/April, the 6-cm variations are faster than the 11-cm variations by a factor of about three. We also note that, from visual inspection of the light curves, there is the suggestion of a time lag of ~1 hour near the minimum at JD 2447255.6, with the 11-cm data lagging behind the 6-cm data (Fig. 1*a* and *b*). A formal cross-correlation analysis of the full data set gives a time lag of 0.5 ± 1 hours. In 1985, 0917+62 was observed twice at 11 cm wavelength[7]; it was variable in both sessions, with amplitudes of 3.4% and 8% respectively.

In contrast to 0917+62, the BL Lac object 0716+71 showed quite different light curves at 6 cm and 11 cm in the March/April dual-frequency run; it is not obvious how these two curves are related to each other. This could imply a time lag longer than the length of our observing period, or it may be that the variations at 6 and 11 cm are indeed unrelated. The light curves of 0716+71 also show significant fine structure on timescales of a few hours, which were not adequately sampled (Fig. 1*d*). In each of the four sessions during which it was observed since 1985, 0716+71 showed a remarkable consistency of behaviour; its variability was always in the narrow range of 7–11%.

We note that rapid radio variability seems to be a quite common phenomenon. It was observed in 6 out of 15 sources by Heeschen *et al.*[7]; three of the sources are classified as quasars and three as BL Lac objects. These sources had been selected on the basis merely of their high declination, flat spectrum, and high flux density. Furthermore, the occurrence of a similar effect—a 7% decrease of the 6-cm flux density in slightly more than 1 hour—in OJ287 (ref. 6) indicates that it is not limited to the north-polar region. On the basis of up to five observing periods now available for several sources, it seems that rapid variability is not distributed randomly among compact sources, but rather that it appears quite regularly in the same sources each time they are observed. Thus this phenomenon is connected either to certain characteristics of the sources (besides compactness, which seems to be a necessary but not sufficient condition) or to their positions in the sky. As is already apparent from the two cases of 0716+71 and 0917+62, the detailed wavelength dependence of the variations—including cross-correlation and possible time lags—is different in the observed sources, but the amplitudes at 6 and 11 cm wavelength are similar.

At least three different mechanisms could explain the observed variability: intrinsic effects, gravitational (micro-) lensing and refraction in the interstellar medium (scintillation).

Although the variability on somewhat longer timescales observed at high frequencies, in 3C279 and OJ287 for example, is connected to variations in the degree and angle of polarization and is therefore generally interpreted as being intrinsic[8,9], it is difficult to attribute variability as rapid as reported here to intrinsic effects. Standard light-travel-time arguments would lead to extremely small source diameters and therefore to brightness temperatures as high as 2×10^{17} to 7×10^{18} K (under the

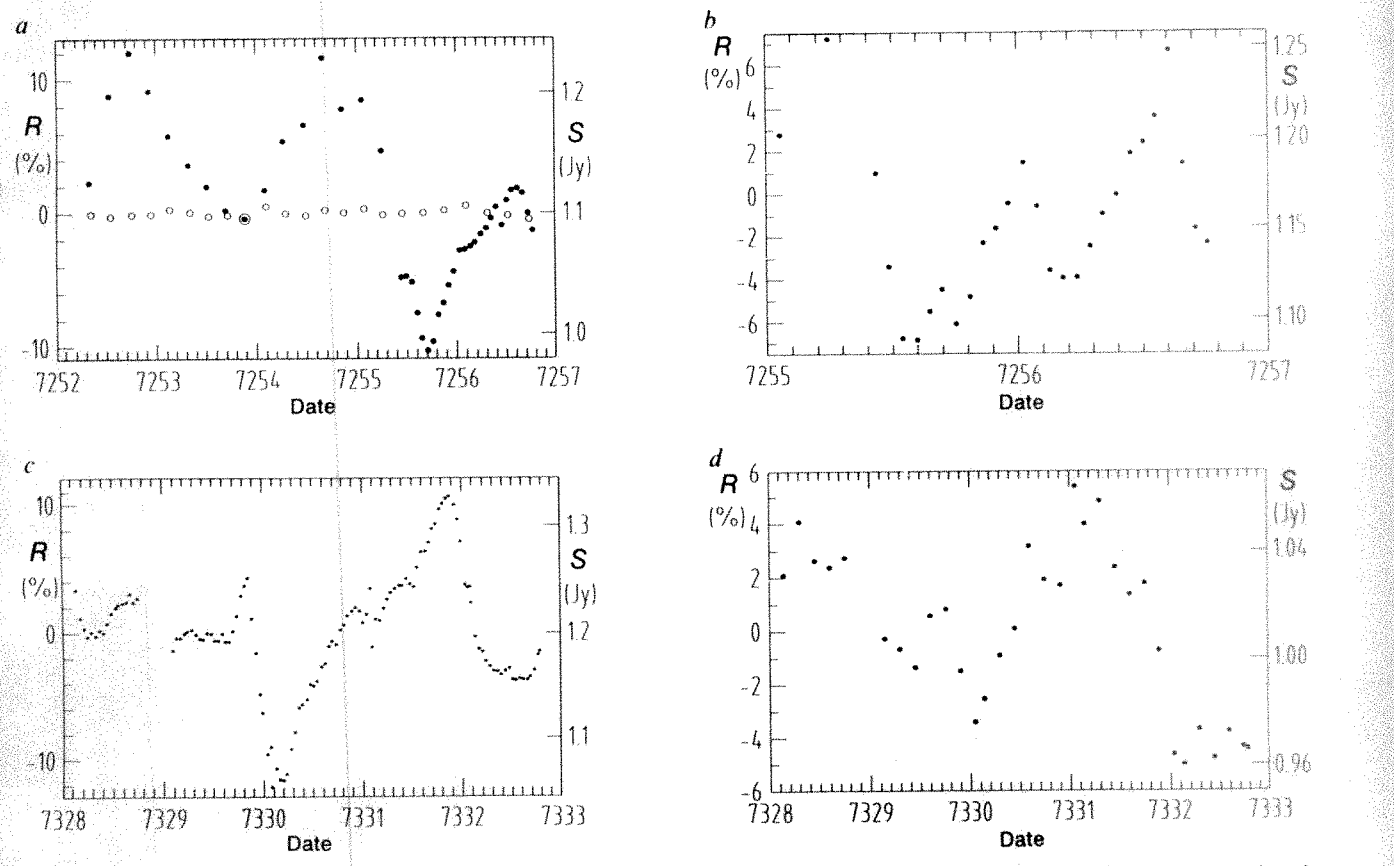


Fig. 1 *a*, Flux density (S) of 0917+62 (filled circles) and the steep spectrum reference source 0951+69 (open circles) at 11 cm wavelength in March/April 1988 against date (JD−2440000). Normalized variations R are defined as 100 ($S/\langle S\rangle-1$). The data for the steep spectrum source indicate the total measurement errors. The absolute scale applies to 0917+62. *b*, Flux density of 0917+62 at 6 cm wavelength, March/April 1988. *c*, Flux density of 0917+62 at 6 cm wavelength, June 1988. *d*, Flux density of 0716+71 at 6 cm wavelength, June 1988.

assumption that the unknown redshift of 0917+62 lies in the range 0.1–1), so that for incoherent synchrotron radiation, Doppler boosting factors of 50–200 would be needed to meet the Compton limit of about 10^{12} K (ref. 7). The same argument applies to scattering by material within the quasar or at cosmological distances: either the source of radiation must be very small, or the apparent relative transverse velocity must exceed ~100c. Coherent mechanisms could also produce fast intrinsic variability, but no convincing evidence for those processes has been found so far. Normally, intrinsic variability is larger at shorter wavelengths, in contrast to the variations observed here, but with the present limited understanding of the relevant physical processes we cannot rule out intrinsic effects as a possible explanation.

If the radiation from a distant quasar passes through an intervening galaxy, one must take into account the relativistic light deflection not only by the whole galaxy but also by individual stars[10]; the combined effects of many stars lead to a distribution of caustics in the source plane. Calculated model light curves look qualitatively very much like those shown here: they show similar discontinuities of the slope, which reflect the convolution of the moving source structure with the distribution of caustics[11,12]; the details depend on the models adopted for the lensing galaxy and the source structure. Gravitational lensing is sensitive to wavelength only through the wavelength dependence of source size and shape, so that it can explain the observed similar variations at 6 cm and 11 cm. Nevertheless, of the above mentioned explanations, gravitational micro-lensing seems the least probable, because we could not find, on the POSS plates, any obvious lens candidates in the fields of 0716+71 and 0917+62. Furthermore, to produce a noticeable effect, the source size cannot be much larger than the variability timescale multiplied by the velocity of light. This leads to about the same source size as required by intrinsic explanations and thus encounters the same difficulties.

Scattering by the interstellar medium is believed to cause low-frequency variability[13,14], as well as flickering at ~10 cm wavelength on timescales of several days to weeks[15,16]. Unusual features in the light curves of compact extragalactic sources sampled once per day[17] are also attributed to scattering by compact structures in the interstellar medium. In the single-scale model, the timescale is simply given by the spatial scale divided by the relative velocity. For galactic velocities of 200–300 km s^{-1} or less, the durations of about a day for single events then imply that the scattering density fluctuations cannot be much larger than ~0.1 AU. The electron density n_e of single scattering blobs can be estimated from $\theta \approx (2\pi)^{-1} r_e \lambda^2 n_e$, where θ is the refraction angle, r_e the classical electron radius and λ the wavelength of the observation. For $\theta \approx 1$ milliarcsec this yields $n_e \approx 10^3$ cm^{-3} and thus a mass of $\sim 2\times10^{16}$ g. At a distance of 1 kpc a scatterer of diameter 0.1 AU covers $\sim 10^{-18}$ sr, so a total mass of $\sim 2\times 10^{34}$ g, or 10 $M_\odot$, is needed to reproduce the observed scattering probability. Although very little is known about the structure of the interstellar medium on scales as small as those involved here, and although electron densities of 10^3 seem quite large, the extreme scattering events[17] give strong evidence for the presence of such clouds. Because of the small timescales present in our data, refractive interstellar scintillation in a medium with a power-law wavenumber spectrum and the standard parameters given by Rickett[18] gives no consistent results, unless the distance to the scattering screen is reduced to ~50 pc. Normally scattering by the interstellar medium is expected to have higher amplitude at lower frequencies, but again the dependence of source size on wavelength may lead to equal amplitudes at different frequencies[19,20], or even to higher amplitudes at higher frequencies, as observed in the extreme scattering events[17]. In this framework,

caustics may also be relevant[21], thus providing an explanation of the observed shapes of the light curves as mentioned above.

Because our sample sources were selected on the basis of their high declination, they all lie in the same region of the sky, which happens to be close to the galactic loop III. It seems possible that the observed variability is connected to this old supernova remnant[7] or to the high-velocity features present in the same area[22,23]. This interpretation is not completely satisfactory, however, because OJ287 shows a similar behaviour[6], but is not located near any known feature resembling the galactic loops.

It is obvious that, irrespective of its reason, variability with amplitudes of several per cent and timescales of hours causes severe problems to aperture-synthesis observations, whose dynamic range may be limited by fluctuations in the source brightness as small as 1% (refs 7, 19). Thus for observers who wish to map low-brightness features in strong sources, to look for weak images in gravitational-lens systems or to investigate clustering of radio sources, it is very important to know in detail the characteristics of rapid variability.

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Slowly moving thermal features on Jupiter

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Obtaining measurements of winds on the jovian planets—Jupiter, Saturn and Uranus—at depths other than the level of the observed visible clouds has long been a problem preventing an understanding of the dynamics of these deep atmospheres[1,2]. Measurements of horizontal thermal structure on Jupiter suggest that the observed zonal (west–east) jet system decays slowly above the visible clouds, persisting over many pressure-scale heights[3]. The ubiquitous visible cloud cover, believed to be composed of ammonia ice crystals lying somewhere between the 700-mbar and 300-mbar pressure levels[4], prevents direct sensing below these levels, so the depth of the wind system remains unknown. Here we report that maps of upper troposphere temperatures derived from Voyager 1 and Voyager 2 infrared interferometer spectrometer (IRIS) spectra show large-scale features which move very slowly; the motion is very different from the inferred local fluid velocity. We suggest that these features may be indicative of a deeply-rooted fluid dynamical regime beneath the surface meteorology, although other explanations are possible.

The Voyager IRIS instruments obtained measurements of the thermal emission of Jupiter over the spectral range 4–55 μm (ref. 5). During the encounters with Jupiter, at a distance of about 3×10^6 km before and after closest approach, the instruments were used to map the planet globally by scanning from north to south along the sub-spacecraft meridian for one rotation of the planet, to produce north–south maps with a spatial resolution of about 10 degrees of longitude at the sub-spacecraft point. Between these sequences, higher-spatial-resolution observations of selected features were obtained. The spectra have previously been inverted to deduce the temperatures at the 270-mbar and 150-mbar pressure levels, the ammonia abundance, and cloud optical depths at 5 and 45 μm (refs 3, 6). Cylindrical projection maps, in the form of digital images, have been created for each of these quantities from the inbound and outbound north–south mapping sequences obtained by Voyager 1 and the inbound map from Voyager 2. Zonal-mean motions as a function of latitude have been evaluated from time-lapse map pairs by using a line-by-line correlation technique previously applied to Voyager imaging mosaics[7]. The velocity resolution for the time interval between the inbound and outbound maps from Voyager 1 is 30 m s^{-1} and for the time interval between Voyager 1 and Voyager 2 is 1 m s^{-1}, based on the IRIS instrument beam size.

Figure 1 displays the correlations between the Voyager 1 inbound and outbound north–south maps of temperature at a pressure of 270 mbar, and of cloud optical depth at 45 μm. The well defined and continuous line of maxima shows that meaningful correlations have been achieved, and this line defines a zonal displacement profile. The most striking difference between the images is the absence, in the temperature correlation image, of the broad region of positive displacement near the equator. Figure 2 shows the displacements corresponding to the maxima from Fig. 1, in units of zonal wind, and the linear-correlation coefficients. Based on a sampling of one observation point every ten degrees, the probability of achieving a correlation coefficient of 0.4 or better for one longitude strip from a random sample of uncorrelated observations is less than 2%. Plotted along with the IRIS results is a zonal-mean-wind profile derived from Voyager imaging-camera data that has been degraded to the same spatial resolution as the IRIS north–south maps. The wind profile from the infrared–optical-depth maps is in good agreement with the visible-imaging profile to within the spatial and velocity resolution limits. In the equatorial region a broad, strong prograde jet exists and is much wider than the spatial resolution of the IRIS beam. Jets observed at higher latitudes with the imaging camera are too narrow to be resolved by the IRIS instrument. Although wide enough, the strong jet at 24° N is not detected because of the lack of longitudinal structure. The results in Fig. 2 indicate that the infrared data is capable of recovering the low-latitude cloud motion field but that the dominant thermal structures do not participate in its motion.

Longitudinal scans from both the ingoing and the outgoing Voyager 1 maps of the 270-mbar temperature field and cloud optical depths at the equator are plotted in Fig. 3. The outgoing scans have been displaced in longitude by the correlation results to compensate for zonal drift and to facilitate comparison of structures. The largest-amplitude thermal structures at 270°, 210° and 60° longitude clearly show the features determining the correlation result. Some weak features do not coincide for the two scans and may drift at the higher rate of cloud features, but enough agreement exists such that identifications can be

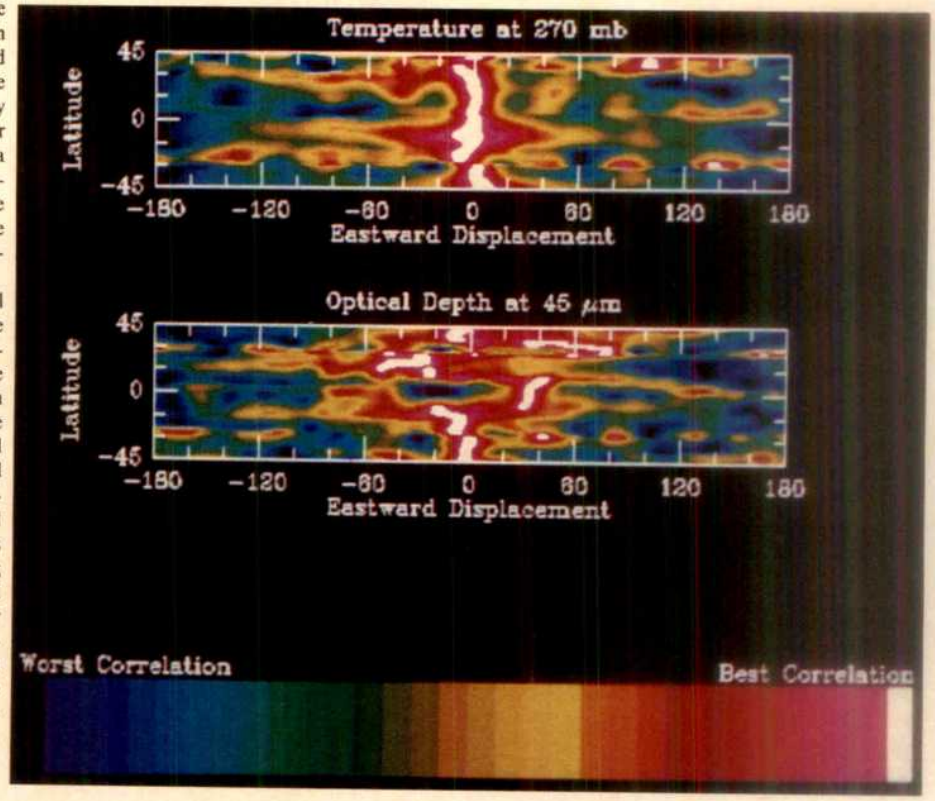


Fig. 1 Correlation images for temperature at 270 mbar (top) and aerosol optical depth at 45 μm (bottom) from Voyager 1 inbound and outbound north–south maps. The value of the correlation function is represented by variations in colour and is displayed for each line of planetographic latitude as a function of eastward longitudinal displacement (degrees) between the lines of the inbound and outbound maps. A positive shift of 30° corresponds to a prograde velocity of ~100 m s^{-1} at the equator.

Method. Cylindrical-projection digital images of the north–south maps used in the correlations were produced by first subtracting the zonal average of the quantity at the latitude of the observation from each measurement. Then the value of the image at each pixel was expressed as a sum of all observations within a given range weighted by a modified gaussian function of the distance between the pixel and the original observation point. This procedure is necessary because the IRIS is a non-imaging instrument. A correlation image is produced by evaluating the correlation function for corresponding lines of a map pair as a function of longitudinal displacement between the lines. For each line of latitude the maximum and minimum correlation-function values are assigned to the corresponding ends of the colour scale.

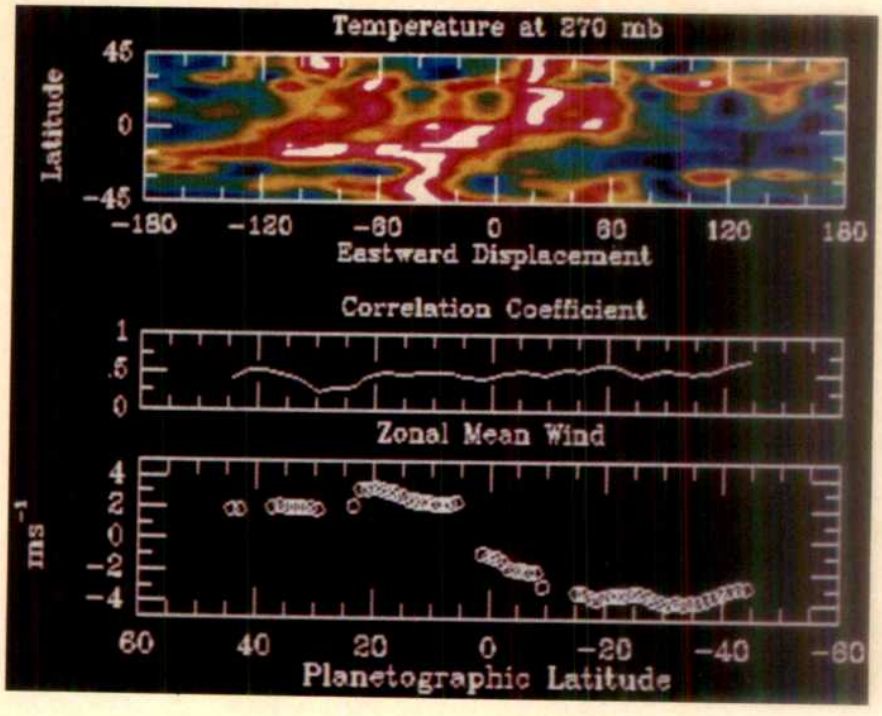


Fig. 4 Correlation results for temperature data (270-mbar level) from Voyager 1 and Voyager 2 inbound maps. Top, Correlation image. Bottom, Zonal velocity versus latitude from the line of maxima in the correlation image. Middle, Linear correlation coefficients from the maxima. See legends to Figs 1 and 2 for further explanation.

made. The small thermal contrasts of the dominant structures are well above the formal noise level of the IRIS. The cloud-optical-depth scans show some structural agreement, although significant changes have occurred; temporal variability in the atmosphere and zonal variation in the wind can lead to such changes. The linear-correlation-coefficient values provide a quantitative measure of the agreement between pairs of scans. The lower correlation coefficients of the optical depths as well as the longitudinal scans in Fig. 3 show that the horizontal cloud structure is more variable than the thermal structure. This result is consistent with Jupiter's pronounced visible variability, which exists in spite of the overall stability of the jet system[1,2].

Further evidence of slowly moving thermal features can be obtained in at least one latitude range. During the closest approach by Voyager 1, Jupiter was longitudinally well sampled between 5° and 15° N at high resolution (about 2 degrees of longitude at the sub-spacecraft point). Fitting a Fourier series to the thermal data retrieved from this sequence, as well as to the corresponding original values in the inbound and outbound north–south maps (that is, before they were made into images), shows a strong component at a wave mode of 9. A least-squares fit of the phase velocity of this wave between all three data sets

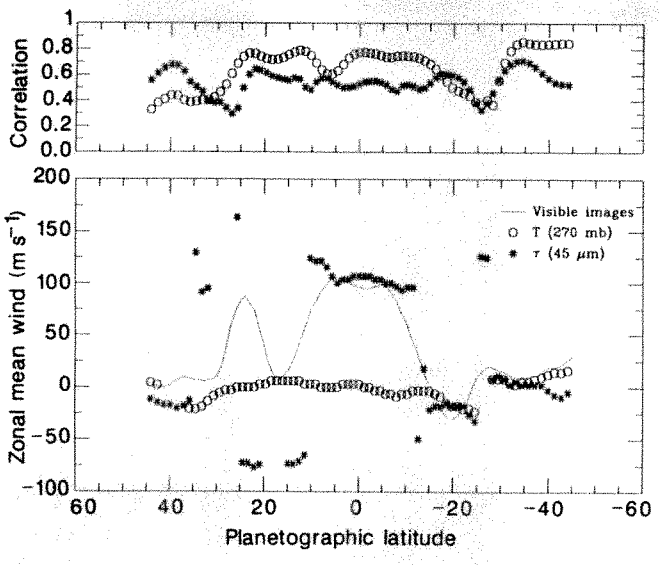


Fig. 2 Zonal velocity versus latitude from temperature (at 270-mbar level), optical depth (at 45 μm) and Voyager 2 violet-image correlations (bottom panel only) (dashed line). The velocities are derived from Fig. 1 by expressing, in units of zonal wind, the displacements associated with the correlation-function maxima. Some missing points are evident at selected latitudes, where spurious correlations occur. Linear-correlation coefficients of the maxima are displayed in the top panel for the infrared results. The visible image velocities are obtained from Voyager 2 violet-image mosaics and have been degraded to the spatial resolution of the IRIS.

yields a value of 10.5 ± 12.4 m s^{-1} (recall that the dynamically important quantity is the phase velocity relative to the mean flow, which, for this range of the total phase velocity, is large and negative).

The high correlation coefficients for the temperature data from Voyager 1 suggest that correlation with the Voyager 2 thermal map might be possible. Moreover, the upper bound on the drift of features provided by the Voyager 1 results indicates that the thermal structures would drift by significantly less than one planetary circumference during the time interval between the Voyager 1 and Voyager 2 encounters (~4 months). Thus, velocities could be obtained unambiguously from the correlations. Figure 4 displays the results of such a correlation. The line of maxima is remarkably continuous and well defined, and suggests that meaningful correlations have been achieved, although the maxima are broader, and the correlation coefficients somewhat lower, than the Voyager 1 results. The line of maxima shows a striking antisymmetry about the equator with an amplitude of ~2-4 m s^{-1}. The velocity corresponding to the maximum at 23° S latitude agrees with the velocity of the Great Red Spot inferred from imaging data[2] to within the formal velocity resolution based on the IRIS beam size (~1 m s^{-1}). A more conservative estimate of the velocity resolution is the width of the half-maximum point of the correlation, which yields a resolution of ±5 m s^{-1}; this resolution is still significantly better than that of the Voyager 1 map correlation. In Fig. 2 the longitudinal scans of the equator show structures at longitudes 270°, 200° and 70° which match the Voyager 1 features, although the matches are certainly not as clear as those in the comparison of Voyager 1 inbound and outbound maps.

The zonal winds in the upper troposphere of Jupiter are believed to be comparable to the winds seen in the clouds, because the vertical zonal-wind shear at these levels is quite small[3]. Although thermal perturbations that drift with the tropospheric currents undoubtedly exist, they are apparently of such small amplitude or horizontal scale that the IRIS beam does not resolve them. The thermal features described here drift slowly with respect to the bulk rotation of the planet as inferred from radio observations[1] and far more slowly than the local aerosol velocity (~100 m s^{-1}). If the thermal perturbations producing these structures are excited in a region where the fluid velocity is comparable to the observed equatorial jet, then the observed motion of the structures would represent a large negative phase velocity with respect to the fluid, and this phase drift would need to have a magnitude sufficient to nearly cancel the fluid motion. Because such nearly exact cancellation of the equatorial jet velocity by the phase velocity seems improbable we believe that the slowly moving thermal features must be excited in a region of slow or no motion. The excitation could result from the influence of dynamical structures in a slowly moving deep atmospheric circulation, from horizontal propagation from slowly moving features at higher latitudes (such as the Great Red Spot), or from upper-atmospheric or magnetospheric features moving with the planetary rotation. We consider the last possibility to be unlikely because of the relatively small energy content of the upper atmosphere. Horizontal propagation may be possible but would need to extend across large horizontal zonal-wind shears. We conclude that the thermal features described here probably represent the influence of slowly moving dynamical structures below the visible clouds on Jupiter.

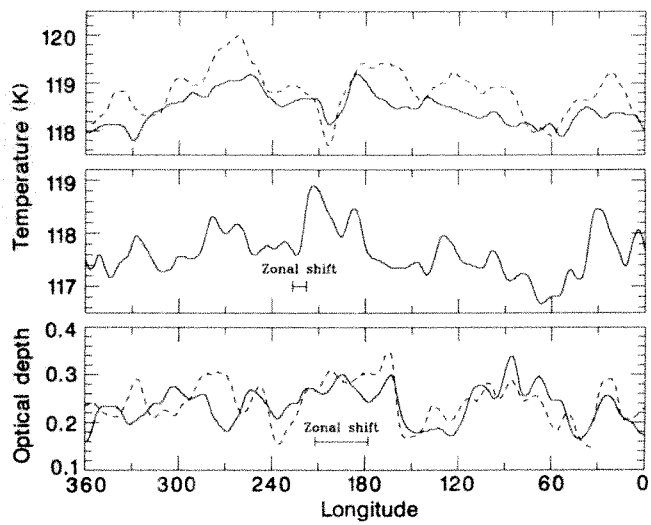


Fig. 3 Longitudinal scans of the equator from Voyager IRIS maps of temperature and optical depth. Top, Scans of temperature from Voyager 1 inbound (solid line) and outbound (dashed line) maps. Middle, Scan from Voyager 2 inbound map. To facilitate comparison of thermal structures, the Voyager 2 scan has been displaced to compensate for zonal drift using the correlation displacement; the size of the shift is displayed. Bottom, Scans of cloud optical depth from Voyager 1 inbound (solid line) and outbound (dashed line) maps. The outgoing map scan has been displaced to compensate for zonal drift using the displacement from the correlation-function maximum in Fig. 2; the size of the shift is displayed at the bottom of the panel.

Previous efforts at inferring the dynamics and structure below the clouds have used wave signatures or flow fields in the visible cloud layers combined with modelling to draw inferences about deep physical properties[9,10]. However, such inferences are somewhat ambiguous and limited to particular regions. These techniques are similar in spirit to seismic studies of the Earth's internal structure and to acoustic-gravity-wave sounding of the Sun[11]. The thermal anomalies reported here may provide a new means of probing Jupiter.

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Direct ozone depletion in springtime Antarctic lower stratospheric clouds

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It is now generally accepted that springtime Antarctic ozone depletion in the atmosphere between 17 and 22 km is related to the presence of ClO produced through heterogeneous chemistry on the surface of polar stratospheric cloud particles[1,2]. Below 17 km, however, where ozone depletion also occurs[3], a causal link with ClO has not been established because of apparently low ClO values. Vertical motions, associated with mountain lee waves and the formation of nacreous clouds, also seemingly cannot explain observed ozone depletion[4]. Here we present balloon measurements of ozone and cloud particles in the lower stratosphere (10–17 km) at McMurdo Station (78° N), Antarctica, on 9 September 1988 which indicate that the cloud particles are directly involved in ozone depletion in this region. The reductions in ozone appear to be correlated with high concentrations of smaller particles, of about 0.2 micrometre radius, which dominate the surface area density in the cloud. The observations suggest a high degree of spatial inhomogeneity of free chlorine, associated with lower stratospheric clouds, and are important in understanding ozone depletion in this region.

Instruments used in the balloon flights included a temperature and electrochemical ozone sensor of high vertical resolution[3], and an individual particle optical counter similar to types used previously in Antarctica[5] but with an air-sample flow rate about 13 times higher ($\sim 200\ cm^{-3}\ s^{-1}$) so as to resolve the low concentrations ($\sim 10^{-3}\ cm^{-3}$) associated with ice crystal formation. The latter instrument measured in seven size ranges, that is, for radii $r \geq 0.20$, 0.25, 0.30, 1.0, 2.0, 3.0 and 5.0 μm, for spherical particles or for slightly non-spherical particles of equivalent volume[5].

Although most flights reached the 30-km altitude region, we concentrate here on the 10–20-km region investigated on 9 September 1988, for which Fig. 1 shows the temperature and ozone data observed. Also shown for comparison are data obtained on 4 September, which are typical of profiles observed before this time. On 9 September the region between 10 and

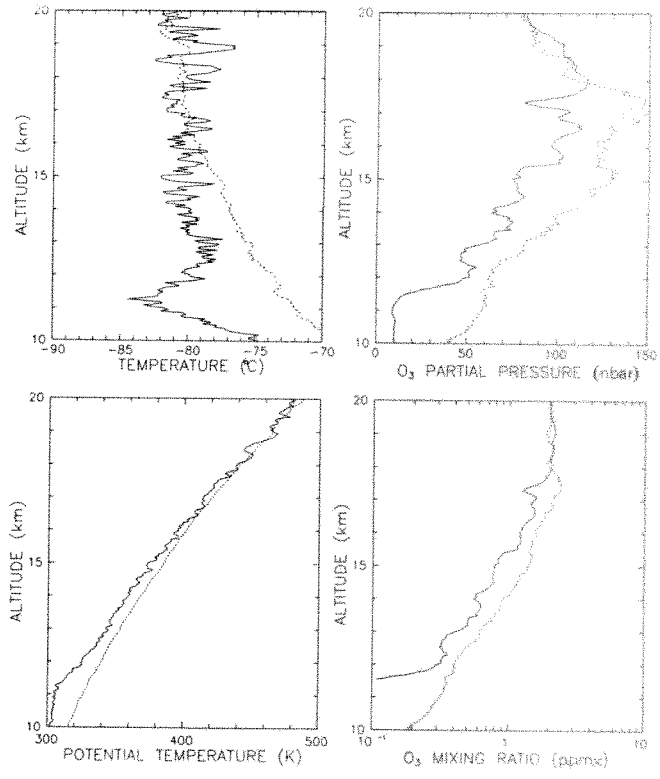


Fig. 1 A comparison of temperature, ozone partial pressure, potential temperature and ozone mixing-ratio profiles measured between 10 and 20 km at McMurdo Station on 4 (dashed line) and 9 (solid line) September 1988.

~11.5 km (an unusually high tropopause) is well mixed and appears to be associated with upward motion; it was in this region that particles with $r \geq 5.0$ μm, presumably comprising water ice crystals, were observed. Above this region, the temperature data indicate a high degree of wave-like stratification with a wavelength of a few hundred metres and an apparent growth in wave amplitude with altitude. The growth rate is consistent with non-dissipative wave propagation, which would double the amplitude between 12 and 20 km.

Reductions in ozone partial pressure at about 12.7, 14.1, 15, 16 and 17.2 km appear similar to those arising from vertical motions, but this is not borne out by the temperature data, as indicated by the potential temperature and ozone mixing-ratio profiles in Fig. 1. Although there exist regions of constant ozone mixing ratio on 9 September, these appear to be unrelated to vertical mixing, because the potential temperature does not show corresponding regions of constancy, as observed in Antarctic mountain-lee-wave events[4]. The region of ice crystal formation on 9 September (10–11.5 km) does show constant potential temperature, associated with cooling of the air parcel by an adiabatic lifting process. The low ozone concentrations in this region are typical of upper-tropospheric values.

The particle measurements made on 9 September are central to an interpretation of the ozone data. Figure 2 shows the 10–20-km profiles of three size ranges, $r \geq 0.20$, 1.0 and 5.0 μm, for 9 September, compared with data obtained with an identical instrument five days earlier. The data for $r \geq 0.20$ μm on 4 September indicate a normal sulphate layer and normal absence of larger particles, as seen in 1986 and 1987[5], except at ~18 km, where there appears to be a slightly enhanced small-particle layer. In contrast, the 9 September profiles are highly enhanced between 11.5 and 17.2 km and between 19 and 20 km; the concentration for $r \geq 0.20$ μm is about 10 times greater than normal at ~12 km. The observations were verified during parachute descent.

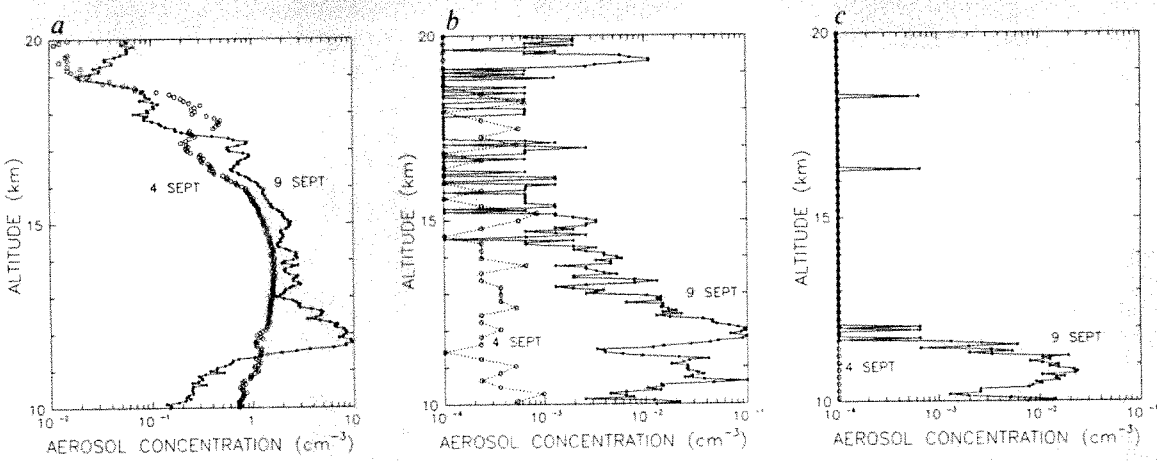


Fig. 2 Aerosol profiles for radii greater than 0.20 (*a*), 1.0 (*b*) and 5.0 (*c*) μm measured between 10 and 20 km at McMurdo Station on 9 September 1988 compared with those measured under non-enhanced conditions on 4 September.

The total aerosol concentration, as observed with a condensation-nuclei ($r \geq 0.01$ μm) counter on 6 and 14 September, was ~20 cm^{-3} at 12 km, so it appears that about half of the available condensation sites grew to sizes larger than 0.20 μm at this altitude on 9 September. The unusual absence of small particles in the 10–11.5-km region is probably associated with attachment to ice crystals in the $r \geq 5.0$-μm layer at this altitude (see Fig. 2). The 1.0-μm profile in Fig. 2 indicates that at 11 km about one half of these particles have radii greater than 5 μm whereas above this altitude (11.5–13 km) all of these particles have radii less than 5 μm and, as the $r \geq 2.0$-μm profile indicates, are essentially all between 1 and 2 μm. Because there is insufficient sulphuric acid vapour to constitute the mass in the 1–2-μm particles observed at 11.5–13 km, and because it is probably too warm (−78 to −79 °C) at this altitude to condense water, these particles may represent the condensation of nitric acid trihydrate (NAT), which can form at temperatures about 7 °C higher than the water ice point[6]. However, if sufficient water vapour was mixed up into the 10–11.5-km region to form water ice at −80 to −84 °C, as indicated by the 5.0-μm data, it is possible that enough water vapour was present at 11.5–13 km to initiate water condensation at these higher temperatures. The lack of vertical mixing above 11.5 km and the abrupt reduction in particle production at this altitude, as indicated by the minimum in 1.0-μm concentration, makes the latter unlikely. Figure 2 also indicates an $r \geq 1.0$-μm layer between 19 and 20 km (another layer was observed between 21 and 22 km) where it would be too dry (frost point ~−90 °C (ref. 7)) for water ice particles to form at the observed temperature of ~−80 °C. Thus, the most reasonable conclusion is that the enhanced aerosol observed throughout most of the stratosphere on 9 September was composed of NAT.

Figure 3 shows the size distributions for the 11- and 12-km data (0.5-km averages) on 9 September, compared with the 4 September size distribution of the normal sulphate layer at 12 km. The enhanced distributions of 9 September are bimodal. At 11 km, the ice crystals have suppressed the sulphate distribution through attachment. Fitting a log-normal size distribution to the large-particle ($r \geq 1.0$ μm) mode at 11 km gives a mode radius of 5 μm, a distribution width of 1.5 and a total concentration of 0.03 cm^{-3}, which results in a surface area density of 13 $\mu m^2\ cm^{-3}$ and a mass mixing ratio, for water composition, of 0.10 p.p.m., which is only a small fraction of the 2–3 p.p.m. of water vapour available[7].

For the bimodal distribution at 12 km, the small-particle mode can be characterized by a mode radius of 0.19 μm, a width of 1.28 and a total concentration of 20 cm^{-3}, which results in a mass mixing ratio of 3.3 parts per 10^9 (p.p.b.). The large-particle mode at 12 km has corresponding values of 0.83 μm, 1.34, 0.3 cm^{-3} and 4.6 p.p.b. Combining the mass in the two modes gives 7.9 p.p.b., which, assuming an NAT composition, implies a value of 2 p.p.b. by volume of HNO_3. Thus the particles in these clouds have probably incorporated a sizeable fraction of the available HNO_3 vapour. Although there is more mass in the large-particle mode at 12 km, the small-particle mode dominates the surface area density (3.1 $\mu m^2\ cm^{-3}$ and 10.2 $\mu m^2\ cm^{-3}$ respectively for the two modes). This suggests that in a typical cloud undergoing particle growth in the lower stratosphere area-dependent phenomena such as heterogeneous chemical reactions are controlled by numerous small particles rather than by a few large particles, in contrast to what has been assumed previously[8]. Thus reductions in ozone owing to heterogeneous chemistry in such clouds should correlate best with the small particle mode.

Figure 4 compares the 10–20-km ozone partial pressure and $r \geq 0.20$-μm aerosol profiles. Negatively correlated layers are apparent at 12.7, 14.1, 15, 16.9 and 17.2 km. Correlations are weak or absent for the 1.0-μm aerosol data, as expected from surface-area considerations. To account for this correlation by quasi-horizontal advection, as has been observed in the past[6], would require a source region unusually rich in particles and poor in ozone. As these observations were made near the centre of the vortex, such ozone depletion cannot be explained by assuming that we are observing low-latitude air; furthermore such enhanced particle concentrations have never been observed in the stratosphere except after major volcanic eruptions and i

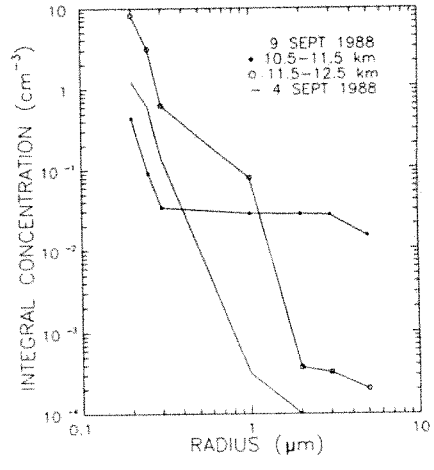


Fig. 3 Integral size distributions for the particle layers observed in polar stratospheric clouds on 9 September and in the undisturbed sulphate layer at 12 km on 4 September.

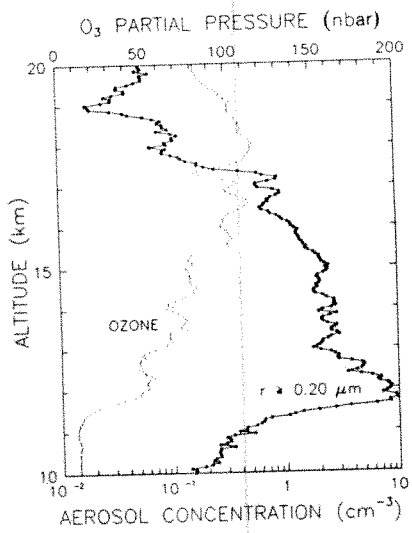


Fig. 4 Ozone partial pressure and $r \geq 0.20$-μm aerosol profiles between 10 and 20 km at McMurdo Station on 9 September 1988. Note the inverse correlations at 12.7, 14.1, 15, 16.9 and 17.2 km.

conjunction with very cold regions of the Antarctic polar vortex[5]. No such eruptions were reported at this time, and particle concentrations had returned to normal values when next measured on 27 September.

These measurements thus constitute the first evidence that ozone is reduced directly within polar stratospheric clouds, in contrast to the comparatively loose correlation assumed previously. This explains why ozone depletion takes place in the lower stratosphere, where the abundance of free chlorine may appear to be too low for conventional ozone depletion chemistry to take place. Temperatures low enough for NAT to exist are present in the lower stratosphere well into October, thus prolonging ozone depletion in this region as observed.

The close anticorrelation with aerosol surface area suggests a direct process whereby ozone molecules are destroyed following collision with particles. Such a process, however, could continue through the winter. As depletion does not begin until early September, photochemistry is clearly involved. It is possible that in the lower stratosphere active chlorine exists only within the denser clouds, in which nitric acid has effectively been removed, whereas above 17 km it exists more uniformly. This may be related to the occurrence of apparently more extensive haze- or veil-type polar stratospheric clouds at higher altitudes, compared to the denser, more inhomogeneous clouds in the lower stratosphere[3,5,7-9]. It would suggest a process that would result in a high degree of spatial inhomogeneity in the ozone distribution of the lower stratosphere and could contribute to the high degree of ozone layering observed in the Antarctic lower vortex[3,4]. Although most of this structure is believed to be related to ozone emanating from the vortex boundary[4], layering in the Antarctic lower-stratospheric ozone distribution has been observed to some extent everywhere within the vortex[3,4,9-11]. This phenomenon may be more readily observable in years when the QBO is in an easterly phase (for example, 1986 and 1988), during which warmer temperatures result in reduced denitrification during the winter.

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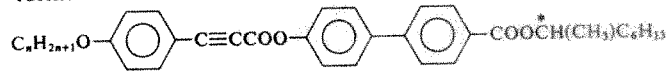
Characterization of a new helical smectic liquid crystal

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The discovery of the first liquid-crystalline material in 1888[1,2] also heralded an age of fascination with chirality and optical activity in ordered fluids. The cholesteric mesophase, which was the first liquid crystal to be found, exhibits form optical activity by virtue of a helical arrangement of its constituent molecules. One hundred years after the discovery of this first liquid crystal, we report the discovery of a new helical smectic liquid crystal, the smectic-A* phase. In this phase the lath-like molecules are arranged in layers with their long axes on average normal to the layer planes. Parallel to the layers there is a helical ordering of the molecules (Fig. 1). We suggest that this phase may be described by a model in which grain boundaries of screw dislocations rotate blocks of layers with respect to each other.

This novel phenomenon was found to occur in an homologous series of ferroelectric liquid crystals, the *R*- and *S*-1-methylheptyl 4′-[(4″-*n*-alkoxyphenyl)propionoyloxy]-biphenyl-4-carboxylates (*n*P1M7). These materials were prepared by the esterification of a variety of 4-*n*-alkoxyphenylpropiolic acids with *R*- or *S*-1-methylheptyl 4′-hydroxybiphenyl-4-carboxylate (ref. 3, and M.A.W. and S.M.S., to be published). The helical A* phase was found in the *n*-tridecyloxy, *n*-tetradecyloxy and *n*-pentadecyloxy homologues, which have the general chemical formula:



and exhibit the phase transitions:

$$\text{Isotropic liquid} \leftrightarrow A^* \leftrightarrow C^* \leftrightarrow S3 \leftrightarrow S4$$

where the new helical phase is denoted as A*, the ferroelectric phase as C* (see Fig. 1)[4], and two further hitherto unclassified helical smectic modifications as S3 and S4 (ref. 5).

Evidence from optical microscopy was obtained for the helical nature of the phase formed first on cooling the isotropic liquid of either the *R*- or the *S*- enantiomers of these esters. For example, in a simple experiment in which the *n*-tetradecyloxy member was sandwiched between clean glass plates, heated to form the isotropic liquid and allowed to cool into the new phase, the phase was found to separate from the liquid in two distinct ways, as shown in Fig. 2*a*. In this photomicrograph the A* phase exhibits two distinct textures, one a platelet texture and the other a Grandjean plane texture[6]. The platelet texture resembles that of Blue Phase I, whereas the Grandjean plane texture appears iridescent and similar to that of the cholesteric phase. As the plane texture nucleates and grows, striations within the texture are seen. These bands possibly correspond to integral multiples of the pitch of the helix of the phase. Subsequent cooling produces a transition to a chiral smectic-C* phase, which exhibits typical banded, focal-conic and iridescent plane

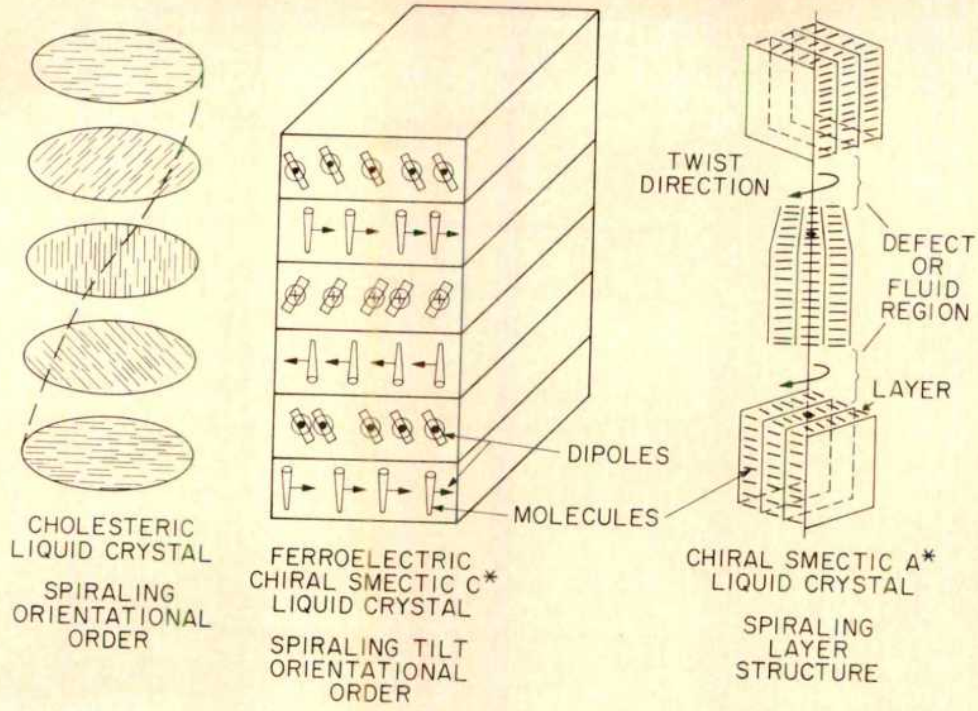


Fig. 1 A pictorial representation of some helical liquid crystal phases, including the cholesteric, the chiral C*, and the chiral A* modifications.

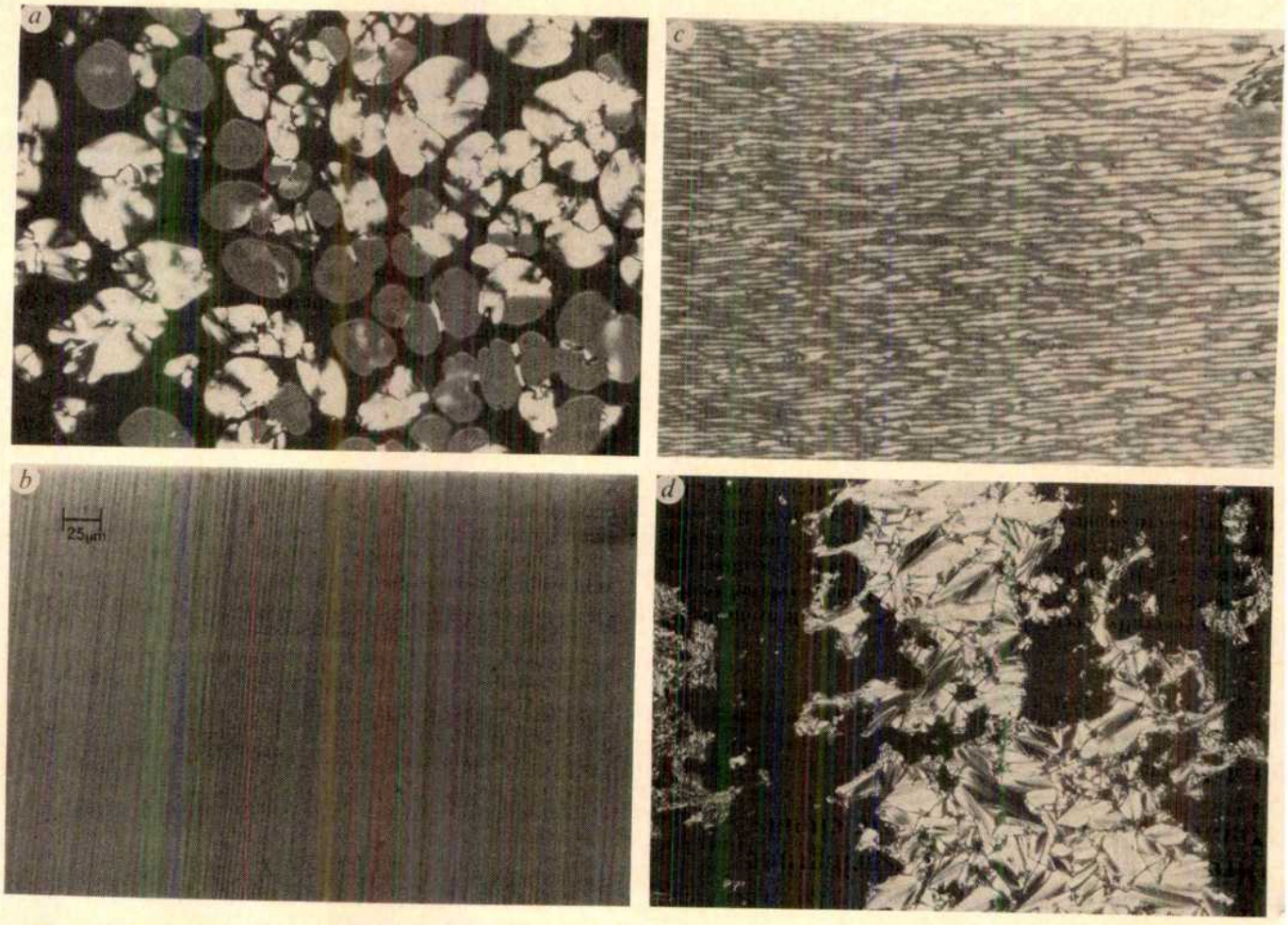


Fig. 2 *a*, The A* phase separating from the isotropic liquid (×100). *b*, An aligned texture of the C* phase of the *R*-tetradecyloxy homologue. The vertical lines are related to the pitch of the helix of the phase. *c*, The same area as in *b*, but the material is now in the A* phase, and the pitch bands are now orientated horizontally. In both photomicrographs, the material was confined in a cell constructed of conducting glass. The plate separation was 10 μm. A holding voltage of 5 V d.c. was applied to the cell in both cases (×100). *d*, A contact preparation between the *R*- and *S*-enantiomers of 1-methylheptyl 4'-[(4"-*n*-tetradecyloxyphenyl) propionoyloxy]-biphenyl-4-carboxylate. The centre of the specimen has the texture of the A phase of the racemic modification, whereas the outer regions have the A* texture of the enantiomers (×100).

textures. The striations on the focal-conic domains, which mirror the positions of the molecular layers, are due to helical pitch bands and dechiralization lines[7]. On cooling through the A* → C* transition, the focal-conic domains of the C* phase grow in the regions of the specimen where the Grandjean texture of the A* phase was present initially. For the Grandjean texture the axis of the helix of the A* phase is normal to the glass plates, but in the focal-conic domains of the C* phase it can be seen, from the pitch bands and dechiralization lines, that the helical axis is approximately parallel to the glass. Thus, if the integrity of the layers is maintained through the phase transition, the helical axes in the A* and C* phases must be mutually perpendicular.

The position of the helical axes in the two phases was confirmed in a separate experiment, in which the material was aligned in a cell constructed from electrically conducting glass. The internal surfaces of the cell were unidirectionally buffed to create sites for planar, homogeneous growth of the liquid crystal[8,9]. Epitaxial growth from the liquid resulted in the formation of a Grandjean plane texture. Rotation between crossed polarizers produced no extinction, indicating that the molecules were symmetrically disposed to the viewing direction. This property is typical of a helical structure[9]. As expected, cooling into the smectic-C* phase produced a focal-conic texture[10]. However, even though the molecules were confined by the aligning agent to lie parallel to the plates of the cell, there was a random orientation of the focal-conic domains. This means that molecules did not lie in a preferred direction within the plane of the cell. This texture is similar to that obtained when the C* phase is formed directly from the cholesteric phase[9].

Application of an electric field to the specimen in the ferroelectric C* phase, for conditions close to the C* → A* transition, produced an aligned sample[9]. In the presence of a small holding d.c. voltage (1 volt per micrometre of cell thickness) dechiralization lines were observed. These lines were oriented at approximately the tilt angle of the C* phase to the buffing direction, and were parallel to the molecular layers (Fig. 2*b*). The axis of the helix is therefore in the plane of the cell and perpendicular to the layers. On heating the specimen into the A* phase, the lines became oriented approximately perpendicular to those in the preceding C* phase, thereby giving a texture reminiscent of the cholesteric finger-print texture (Fig. 2*c*). This confirms that the helical axes in the two phases are oriented perpendicularly. Thus, the helix lies in the plane of the layers in the A* phase. The two relevant textures are shown in Fig. 2*b* and *c*, in which are displayed equivalent areas for each phase. When the holding voltage is relaxed, the A* phase reverts to a plane texture.

For the purposes of phase classification, the new A* phase can be clearly distinguished from the cholesteric phase in two different experiments. One of these examines the properties of the racemic modifications of these compounds and the other probes the structure of the phase by X-ray diffraction.

The racemic modifications of these materials can be obtained either by synthesis from racemic starting materials or by mixing the *R*- and *S*-enantiomers. In the first case the synthetically prepared material was found to exhibit normal A and C phases. In the second, the liquids of the *R*- and *S*-enantiomers can be sandwiched between glass plates as before and cooled into their liquid-crystalline phases[11] (Fig. 2*d*). At the junction of the two materials, a racemic modification appears. The proportions of enantiomers in the mixture change with distance from this interface so as to eventually give one or other of the pure enantiomers. Thus a concentration gradient can be observed in the microscope, and the phase changes that occur with varying concentration can be studied as a function of temperature. At the interface, the racemic mixture exhibits the A phase, which was identified from its focal-conic texture. Moving away from the interface there is a continuous change in texture from a homeotropic to a platelet texture as the A* phase appears (Fig. 2*d*). As there are no abrupt changes in the texture to indicate

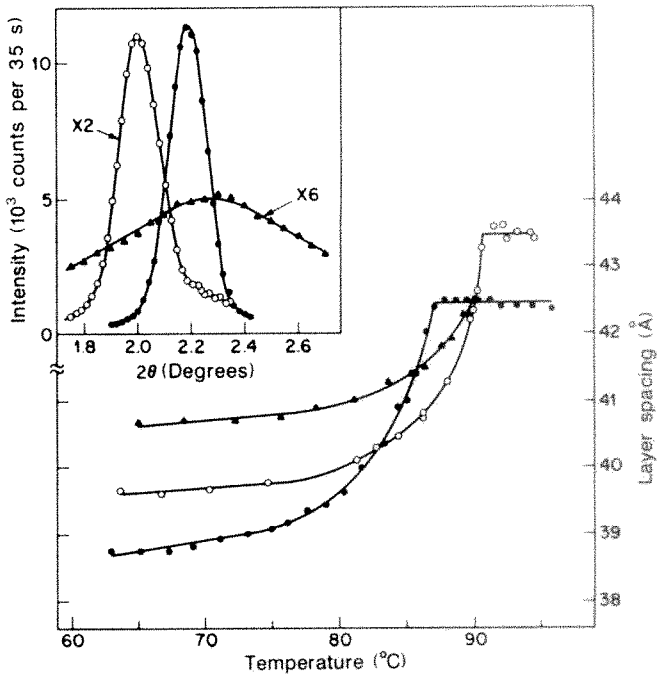


Fig. 3 The temperature dependence of the layer spacing for the $n = 12$ (filled circles), 14 (open circles) and 16 (triangles) *R*-enantiomers. The inset shows scans through the layer peak for the $n = 14$ enantiomer at temperatures within the C*-phase (closed circles; $T = 82.8$ °C), the A*-phase (open circles; $T = 93.3$ °C), and (just into) the isotropic-phase stability fields (triangles; $T = 97.4$ °C).

a phase change, this observation suggests that the A and A* phases are continuously miscible[12], thereby classifying the helical phase as A. It is also interesting to note that the A phase formed at the interface of the racemic mixture has a higher transition temperature than the surrounding corresponding pure enantiomers. Thus, the A phase of the racemate appears to be more stable than the A* phase of the enantiomers. Cooling resulted in a transition to the respective C and C* phases.

Further evidence for distinguishing the A* phase from a cholesteric phase was obtained from X-ray diffraction using an 18-kW rotating-anode-based spectrometer. For this study, unaligned samples of the *R*-*n*-dodecyloxy, *R*-*n*-tetradecyloxy and *R*-*n*-hexadecyloxy ($n = 12$, 14, 16) homologues were prepared in 1-mm glass capillaries. A vertically bent pyrolytic graphite (002) crystal was used to focus Cu Kα X-rays to a 0.5×2.0 mm spot on the sample. The scattered radiation was then analysed using slits and a flat pyrolytic graphite crystal. This defined an instrumental resolution of 0.01 Å^{-1} full width at half maximum in the scan direction, and 0.02 Å^{-1} transverse to the scattering plane.

The primary X-ray study consisted of 2θ scans through the smectic layer peak. The temperature dependence of the layer spacing for the three enantiomers is shown in Fig. 3. The layer spacing for each of the enantiomers increases continuously on heating and, for the *n*-dodecyloxy and *n*-tetradecyloxy members, saturates in the A* phase at a relatively constant value. The lamellar spacing was found to be commensurate with the length of a fully extended molecule.

The inset of Fig. 3 shows layer peak scans taken within the C*, A* and isotropic phases of the *R*-*n*-tetradecyloxy homologue. Within instrumental resolution, the layer peak widths for the C* and A* phases are similar, and the integrated intensities in these peaks differ by only a factor of two. This implies that the A* layer correlations extend a minimum distance of ten layers, thereby establishing smectic layering as a key structural element of this phase. At a temperature just above the A* → isotropic-liquid transition, a diffuse layer peak is seen with a peak position that is comparable to the layer peak position measured well within the temperature range of the C*

phase. The nature of this observation will be discussed briefly later.

Thus, the qualitative model that emerges for this new phase is one in which the elongated molecules are arranged in loosely ordered layers with their long axes either normal to or only slightly tilted with respect to the layer planes. In the direction of the layer planes there is a twist in the parallel orientation of the molecules. This twist can be effected either by defects, such as screw dislocations, between short sections of the A phase, or by cholesteric liquid-like regions separating larger smectic-A domains, as shown in Fig. 1. The full structural details of this phase are now being studied by high-resolution X-ray diffraction from aligned samples and by freeze-fracture studies. The results obtained from these investigations will be reported soon.

Finally, we note that de Gennes[13] has predicted that, just as magnetic flux can penetrate Type II superconductors in a lattice of vortices, twist or bend distortions can be incorporated into a layered A structure by the presence of an array of screw or edge dislocations. A model for such an array was proposed recently by Renn and Lubensky[14]. With reference to the structure of the A* phase shown in Fig. 1, their model specifies that grain boundaries of screw dislocations are responsible for rotating the individual 'blocks' of the A* layers with respect to each other. They call the resultant phase a twist-grain-boundary (TGB) phase, and predict that it can occur between the cholesteric and the A phase in a manner similar to the occurrence of the Abrikosov flux-lattice phase between normal and superconducting phases[15]. Renn and Lubensky established that the requirement for this intermediate phase to occur is that the Ginzburg parameter, which is equal to the ratio of the twist penetration length (λ_T) to the smectic-A correlation length (ξ), must be greater than $1/\sqrt{2}$.

Consequently, the TGB phase is a feasible model for the A* phase, for the following reasons. First, the X-ray scattering pattern predicted for the TGB phase is consistent with our X-ray measurements on the A* phase. Second, the model predicts a helical structure with the helical axis parallel to the smectic layers, which is consistent with our optical experiments. Finally, we observe the A* phase in the vicinity of an A→C* transition, where the A phase is stable over a short temperature range. At such a transition, λ_T diverges, resulting in a large value for the Ginzberg parameter, as required by the theory (R. B. Meyer, personal communication). Assuming that the TGB phase is a viable model for the A* phase, we can explore the possible implications of this phenomenon still further. In particular, we can speculate on the nature of the effect observed near to the clearing point in the isotropic liquid. For the superconducting flux lattice it has been proposed[16] and observed[17] that the vortex lattice can disorder into an entangled or disentangled fluid of vortices. We suggest that a similar disordering of the regular array of screw dislocations in the TGB phase may be the mechanism responsible for the occurrence of the effect observed in the isotropic liquid at a temperature just above the transition from the A* phase.

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Disequilibrium of Holocene sediment yield in glaciated British Columbia

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It is generally supposed that specific sediment yield—the quantity of sediment passing a monitored river cross-section per unit area drained upstream of that section—declines as the area drained increases[1–3]. Part of the sediment mobilized from the land surface is supposed to go back into storage at field edges, and on footslopes and floodplains. In contrast, we show here that data from British Columbian rivers reveal a pattern of increasing specific sediment yield at all spatial scales up to 3×10^4 km^2. This results from the dominance of secondary remobilization of Quaternary sediments along river valleys over primary denudation of the land surface. The result controverts the conventional model which has been derived from studies of small, highly disturbed agricultural catchments. The rivers are still responding to the last glaciation, giving a landscape relaxation time greater than 10 kyr. This holds profound implications for geomorphological theory and for studies of erosion.

Happ and others[4–6] have shown that specific sediment yield presently increases downstream in the south-eastern United States, which they explained in terms of remobilization of sediment eroded from uplands during the first two centuries of European agrarian settlement. Schumm[7] has asserted that such a complex response to episodes of upland erosion is the usual pattern of river-basin development, but apart from a speculation by Meade[8] that the response in this region may persist for centuries, little has been said about the timescales of adjustment of the fluvial landscape to perturbations in sediment supply, nor has the conventional model been tested critically in natural landscapes.

Our main data resource is the archive of suspended sediment loads obtained by the Water Survey of Canada since 1965. These data (Fig. 1) have been discussed at length elsewhere[9,10]. Here we emphasize only that they represent contemporary fluvial sediment transfers in British Columbian rivers. Bedload transport, which is not normally measured, accounts for a comparatively small proportion of the total load[11]. Although solutes may be significant, they do not alter the pattern presented in Fig. 1[9]. The data have been augmented by results from research studies of small drainage basins[9,12], which allow us to extend the domain of drainage areas by two orders of magnitude. We have classified basins according to whether (1) they contain contemporary glaciers, (2) they contain large lakes or reservoirs, (3) they have been disturbed by human land use, including mining and contemporaneous clearance of more than 5 per cent of the forest, and (4) they are undisturbed. No basin has a significant proportion (>5%) of arable land.

The relation of sediment yield to drainage area (Fig. 1) is complex, but a clear trend emerges for basins that are classified as undisturbed. Only three such basins plot outside this trend. Specific sediment yield increases through all areas up to about 3×10^4 km^2. Between 40 km^2 and 10^4 km^2, specific yield is approximately proportional to (area)$^{0.6}$, implying a near-linear increase in sediment yield downstream. The local range of the main trend is between about one-half and one order of magnitude in yield: detailed examination of the data[10] indicates that variable Quaternary and bedrock geology is the cause. There is no systematic association, however, between rock type and topography to explain the overall trend, which is replicated within individual major drainage basins.

On the evidence of Fig. 1, primary denudation, indicated by sediment yield in the smallest basins, is more than an order of

Table 1 Comparison of predicted and measured streambank erosion

River	h (m)	M (m yr^{-1})	dY/dL* (Mg m^{-1} yr^{-1})	A_d (km^2)	Predicted range of dY/dL†	Comments
Liard and Peace River systems‡						
Sikanni Chief	8.3	2.9	13.8	10,530	7.0–38	Sand bed
Fontas	6.5	2.9	9.9	8,110	5.4–30	Sand bed. M assumed
Muskwa	4.0	2.7	4.0	1,510	0.8–4.1	Gravel bed
Prophet	6.0	2.3	7.0	6,765	5.3–29	Gravel bed
Fort Nelson	7.0	4.4	16.5	47,815	32–173	Sand bed. A_d > limit
			(From actual envelopes in Fig. 1:		16–87)	of increasing yields
Beatton	7.0	0.5	1.9	4,070	1.8–10	Fine gravel. M from Nanson[23]
Upper Fraser River system						
Upper Eagle	3.5	1.3	1.5	150	0.10–0.6	Gravel bed. Disturbed?
Lower Eagle	5.0	0.7	1.6	1,385	0.6–3.3	Sand bed

h = mean bank height; M = mean meander migration rate on bend axis. Data from refs 14, 15.
* Calculated as 1.5 $(h-2)M/2$; 1.5 (Mg m^{-3}) is the assumed bulk density of bank material, $h-2$ adjusts for bed material in the lower bank, and $M/2$ gives the average erosion rate assuming linear variation around the bend.
† From equations in the text; equation for mainstream length is $L = 1.2\,A_d^{0.6}$
‡ Some of these rivers are identical with ones in our study, but the places of measurement are different.

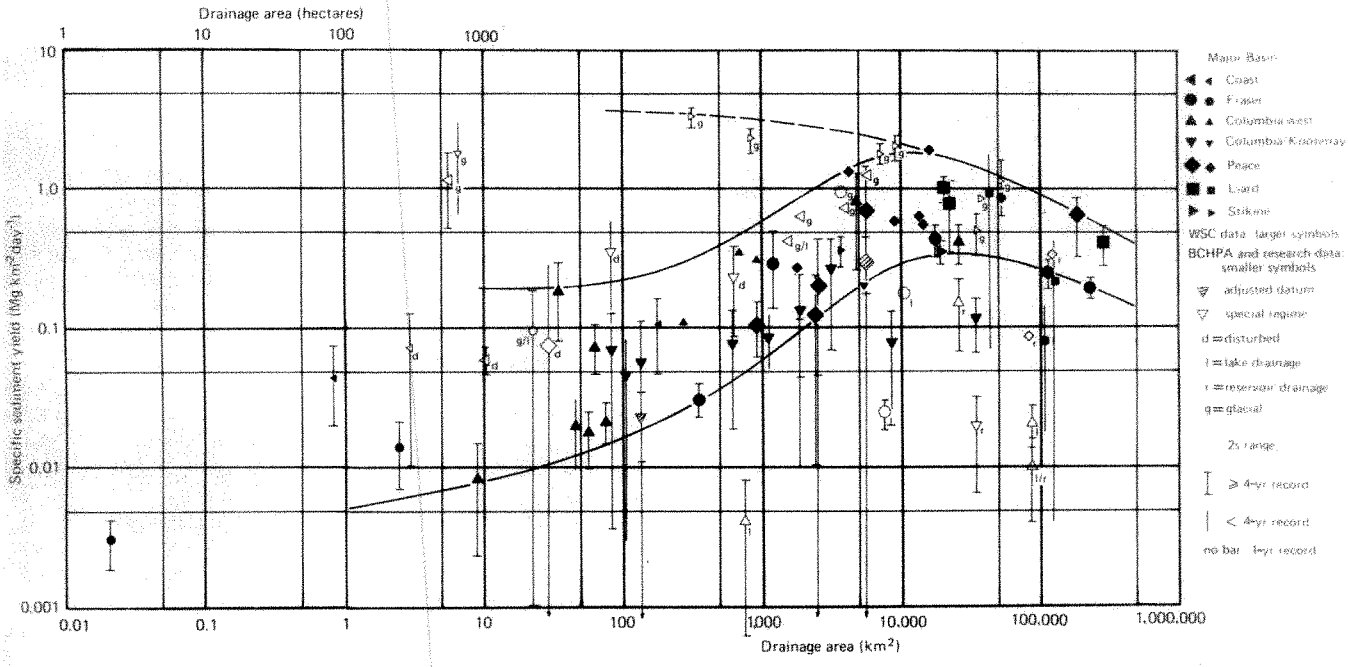


Fig. 1 Specific sediment yield as a function of drainage area for fluvial suspended-sediment-transport records in British Columbian rivers. Closed symbols indicate data for the natural landscape; open symbols indicate yields influenced by natural or human disturbance factors. The main trend of undisturbed yields was sketched by eye. See ref. 10 for a detailed discussion of the data.

magnitude smaller than yield rates in medium to large basins. The latter include a large component arising from secondary remobilization of recent sediments[13]. One must conclude that most of the sediment transported in British Columbian rivers is recruited from the riverbanks and immediate valley-sides. Much of it is derived from the erosion of Quaternary sediments in the valleys and from landsliding of incompetent valley walls.

Thus, the rivers are degrading through valley fill deposits. This is qualitatively evident by the widespread occurrence of Holocene terraces and entrenched trunk streams. We are able to make a quantitative test of our conclusion using data on rates of meander migration obtained by Hickin and Nanson[14,15]. Assuming a linear relation between specific sediment yield and mainstream length (L) for the rising limb of our trend, total sediment yield Y is proportional to $A_d L$, where A_d is drainage area, so that dY/d$L \propto A_d$. The upper and lower envelopes of our plot are then given approximately by dY/d$L = 36 \times 10^{-4}\,A_d$ and dY/d$L = 6.5 \times 10^{-4}\,A_d$, with the coefficients appropriate for units of Mg m^{-1} yr^{-1}. dY/dL is the rate of increase in net erosion downstream, or the net rate of bank erosion if the sediment comes from the stream banks. Table 1 shows that the measurements of Hickin and Nanson generally fall within the range predicted by our analysis. All the results demonstrate that riverbank erosion rates are sufficient to support our interpretation of the regional trend of increasing sediment yield.

The conventional model, in which specific sediment yield decreases with basin area, was derived mainly with reference to intensely cultivated regions in which the land surface is highly disturbed and exposed to erosion[1,2], so that headwater sediment supply is comparatively unlimited. The sediment yield pattern observed in the natural landscape of British Columbia is more consistent with common notions of pristine headwater streams—particularly in mountains—and sediment-laden trunk rivers. A pattern of sediment yield similar to that observed here has been

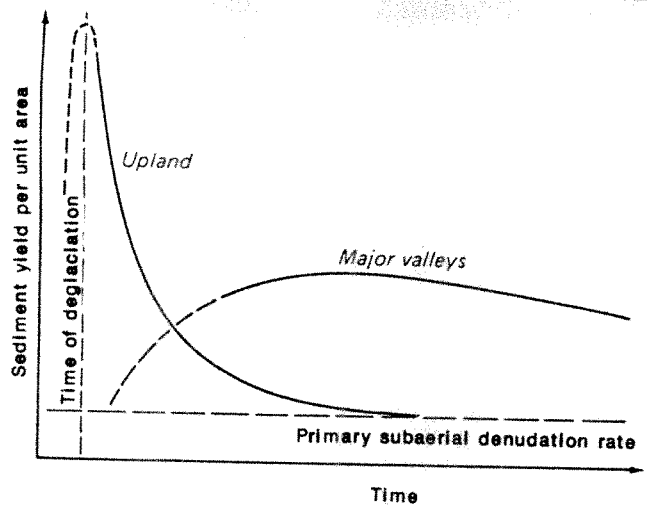


Fig. 2 The paraglacial sedimentation cycle[17], modified to indicate the effect of spatial scale upon the temporal pattern of sediment yield. The time axis spans approximately 10,000 yr.

reported on the Canadian prairies[16], with similar rationalization.

The major rivers are still redistributing sediment that was delivered to the valleys by glacial events more than 10 kyr ago. The transfer and disposition of this material has been termed a 'paraglacial cycle' of sedimentation[17]. Originally, evidence of a paraglacial cycle was adduced from outwash deposits and alluvial fans present in the major valleys, and a relaxation time of a few thousand years was inferred from chronostratigraphic markers in the fans. It is now apparent that this timescale is relevant only for the restricted spatial scale of the upland basins that deliver sediment to the fans. An extended paraglacial cycle (Fig. 2) may be said to dominate still in the larger basins. The trend of specific sediment yield in the largest basins ($>3\times10^4\,km^2$) is consistent with that of contemporary glacierized basins—the most disturbed basins in the region—and conforms approximately with the conventional model. The largest basins are still responding to glaciation. The timescale for ultimate dissipation of this phenomenon appears to be many tens of thousands of years.

It has been supposed for more than 30 years that landscapes adapt so as to reach equilibrium, in some sense, with the imposed environment[18,19], which, for stream systems, implies the hydroclimate[3]. Sediment yield is thereby supposed to be the consequence of erosion from the surface of the land and is supposed to reflect the denudation rate for the prevailing climate and regional geology. This view has been challenged[20] on the basis of observations in the south-eastern United States[4,5]; it certainly is not true at any resolved scale in British Columbia. Fluvial sediment yield at all scales above 1 km^2 remains a consequence of the extraordinary glacial events of the Quaternary Period, rather than of Holocene erosion of the land surface. The results presented here confirm Schumm's contention[7] that fluvial sedimentation does not reflect contemporary erosion of the land surface at any timescale below that for significant evolution of the entire land mass, which is of the order of several ten thousands of years. The natural landscape of British Columbia is imprisoned in its history.

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Circadian rhythm and light regulate opsin mRNA in rod photoreceptors

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Disk membranes in the outer segment of rod photoreceptors are continuously renewed, being assembled at the outer segment base, displaced outward by new disks and eventually shed at the tip[1]. In lower vertebrates, disk assembly occurs with a diurnal rhythm with 2–4% of the outer segment length produced daily[2,3]. We have discovered that in toad and fish retinas the level of mRNA for opsin, the most abundant protein in rod disks[4], fluctuates with a daily rhythm and is regulated both by light and by a circadian oscillator[5]. The mRNA level rises before light onset, remains high during the light phase of a diurnal cycle and decreases four to tenfold during the dark phase. In constant darkness, mRNA elevation occurs during subjective daytime. At night, rod opsin mRNA can be elevated by exposure to light.

The level of rod opsin mRNA was measured by hybridization of an opsin antisense RNA probe to blots of total RNA isolated from the retinas of toads (*Bufo marinus*) and fish (*Haplochromis burtoni*). The opsin RNA probe was synthesized *in vitro* using a fragment subcloned from the bovine rod opsin cDNA (Fig. 1). This fragment is highly conserved in both human[6] and *Drosophila melanogaster*[7] rod opsin, but badly conserved in the rod opsin homologous proteins: β-adrenergic receptor[8] and muscarinic acetylcholine receptor[9]. The use of rod opsin probes across species is also justified by the homology between the visual pigment genes in species ranging from archibacteria to mammals[10].

The specificity of our antisense opsin RNA probe, FH97, in toad and fish tissue was verified by hybridization both to blots of total retinal RNA and *in situ*, to retinal sections. At high stringency hybridization (10–12 °C below the calculated transition temperature for the hybrid assuming perfect nucleotide matching[11]) a single band was detected in autoradiograms of both toad and fish RNA blots (Fig. 1*a*). In toad RNA blots, a fainter, smaller band was occasionally detected. The labelled RNA was 2.85 kilobase (kb) in the toad and 1.4 kb in the fish, suggesting that in toads, but not fish, the rod opsin mRNA includes an untranslated sequence. *In situ* hybridization at identical stringency revealed that the reactive RNA was located exclusively over the myoid region of the photoreceptor inner segments (Fig. 1*b*, *c*).

To determine whether both rod and cone-opsin species were detected by FH97, we used *in situ* hybridization of dark adapted fish retina in which the cone inner segments are displaced relative

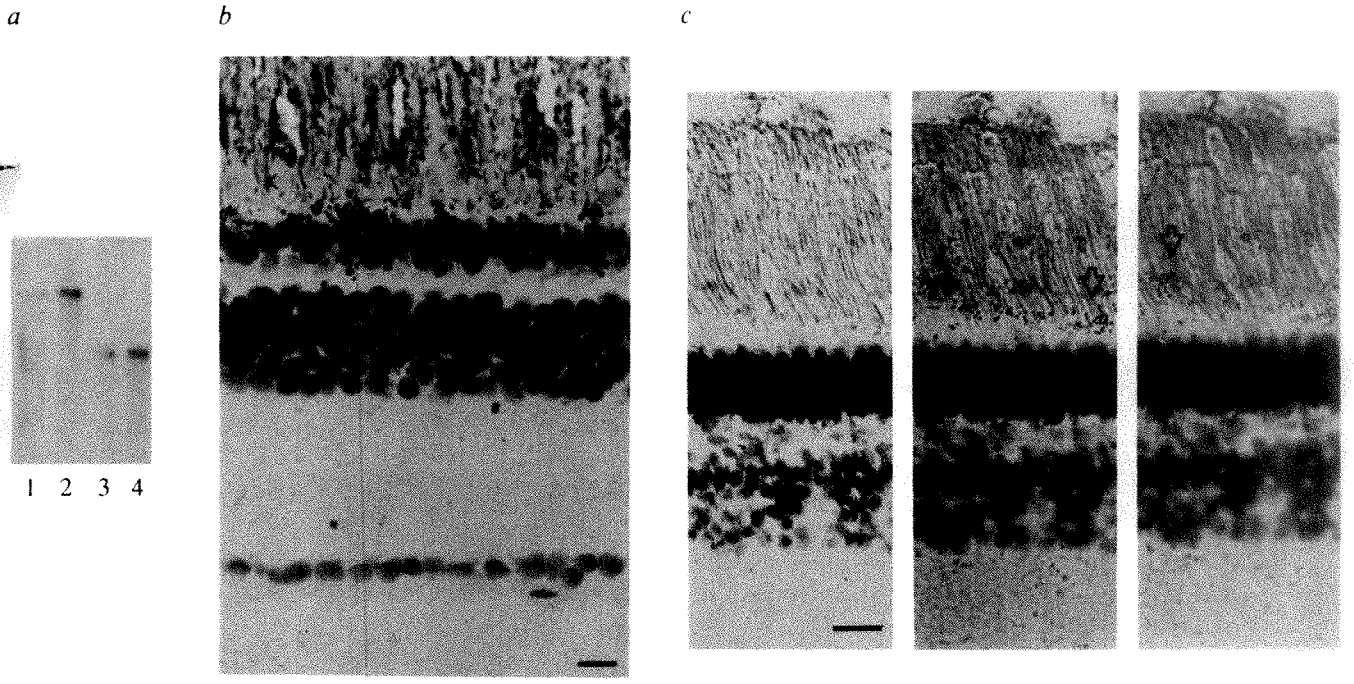


Fig. 1 *a*, Identification of rod opsin mRNA in total retinal RNA blots of toad, *Bufo m.*, and fish, *Haplochromis b.* At high stringency, only a single band was detected in autoradiograms from both toad (lane 1, 4 μg; lane 2, 8 μg) and fish (lane 3, 5 μg, lane 4, 10 μg). *b*, *c*, *In situ* hybridization of retinal sections from toad (*b*) and fish (*c*) with ^{35}S-labelled FH97 antisense mRNA probe. Hybridization was conducted at the same stringency used to probe the RNA blot in *a*. *b*, A light-microscope autoradiograph of a light-adapted toad retina. Autoradiographic grains are exclusively localized over the inner segments of the photoreceeptors. *c*, Three photomicrographs of the same field from a dark-adapted fish retina. The left micrograph is focused on the cell bodies, whereas the middle and right micrographs are focused at two different depths within the autoradiographic emulsion. In the middle micrograph, the autoradiographic grains (arrow) are seen distributed over the inner segments of rods. In the right micrograph, the grains (arrow) are localized in discrete groups over the inner segments of cones displaced from the rod inner segments. Scale bar, 10 μm.

Methods. *a*, Retinas and adhering pigment epithelium were isolated, frozen and maintained in liquid nitrogen until processed. Total RNA was isolated and purified[19], separated by size in a denaturing gel (formaldehyde) and transferred to Nylon 66 membranes[20]. Blots were prehybridized at 55 °C in 50% formamide, 5 × SSC, 5 × Denhardt's, 1% sarcosyl, 5 mM EDTA, 0.1 mg ml^{-1} yeast tRNA, 0.1 mg ml^{-1} sonicated salmon sperm DNA. Hybridization was at 55 °C for 20 h in the same solution with 10^6 c.p.m. ml^{-1} of ^{32}P-labelled probe. Final stringency of hybridizataion was obtained by washing at 75 °C in 0.2 × SSC with 2.5 mM sodium pyrophosphate, 10 mM EDTA and 0.1% sarcosyl. The opsin antisense mRNA probe (FH97) was synthesized *in vitro* (pGEM4, Promega) from a fragment of bovine rod opsin cDNA obtained by *Fnu*DII–*Hin*fI digestion (nucleotides 544–641) of a full cDNA clone[10]. This 97 base pair (bp) fragment (FH97), codes for amino acids in the intra-membrane helix V and the loop between helices IV and V on the intra-discal surface of rod opsin[21] *b*, *c*, Eyes were fixed by immersion for 50 min in 4% paraformaldehyde, 0.1% glutaraldehyde in 0.1 M phosphate buffer, *p*H7.4 followed by 4% paraformaldehyde in the same buffer for 15 min. Tissue was embedded in diethylene glycol distearate (DGD)[22]. Retinal sections (3 μm thick) were placed on poly-L-lysine (0.1 mg ml^{-1}) coated slides and DGD removed. Sections were rehydrated, acetylated[23] and prehybridized for 1 h at 45 °C in 60% formamide, 1 × Denhardts', 75 mM NaCl, 5 mM Tris *p*H 8, 2.5 mM sodium pyrophosphate, 5 mM EDTA, 0.1 mg ml^{-1} yeast tRNA, 0.1 mg ml^{-1} denatured salmon sperm DNA. The sections were hybridized for 6 h at 45 °C with ^{35}S-labelled antisense RNA FH97 added to the same medium in the presence of 100 mM dithiothreitol. After hybridization, sections were washed with 500 mM NaCl, 30 mM Tris *p*H 8 at 45 °C, incubated with RNAase A in this solution at 37 °C for 30 min and washed extensively in 500 mM NaCl, 30 mM Tris *p*H 8 at 45 °C. Slides were coated with NTB-2 emulsion (Kodak), exposed at 4 °C, developed and stained with cresyl violet.

to those of the rods[12] and found that the probe labelled both rod and cone inner segments (Fig. 1*c*). Although FH97 antisense RNA probe could identify both rod and cone mRNA, in blots of total retinal RNA rod opsin mRNA is almost certainly more abundant than cone opsin mRNA. In toads, for example, there are threefold more rods than cones and each rod outer segment is about 30-fold larger than that of a cone. If both cells renew outer segment membranes at the same proportional rate, rod opsin mRNA would be 90-fold more abundant than cone opsin mRNA. For fish, the same reasoning suggests a 20-fold excess of rod over cone opsin mRNA. To test this directly, we hybridized blots to an antisense RNA probe synthesized from either the red or blue human cone opsin cDNA[13]. Under the stringency conditions at which FH97 revealed a single band, neither cone probe yielded any detectable signal, although several bands, including that marked by FH97, were labelled at lower stringencies (data not shown). This failure to detect any bands at high stringency suggests that the amount of cone mRNA present in our standard retinal RNA blots is below our ability to be detect it, as the numerical arguments above suggest.

Rod opsin mRNA abundance during a diurnal cycle was measured in the retinas of animals maintained in 12-hour light–dark cycles and killed every three hours starting half an hour after light onset. In both toads and fish, the mRNA level during the light phase of the cycle was nearly constant and decreased promptly fourfold to tenfold at night (Fig. 2).

Diurnal variation of rod opsin mRNA level could be a response to the light periodicity, or reflect endogenous, circadian signals. Such rhythms are recognized when the time-locked phenomenon occurs periodically in the absence of overt light–dark cycles. We measured opsin mRNA in toads that, after a 12 h : 12 h light : dark cycle, were then kept in darkness and killed at three-hour intervals beginning half an hour after the onset of subjective daytime (Fig. 3*a*) or half an hour after the beginning of the second subjective day (Fig. 3*b*). The rod opsin mRNA levels in these animals were nearly identical to those of animals

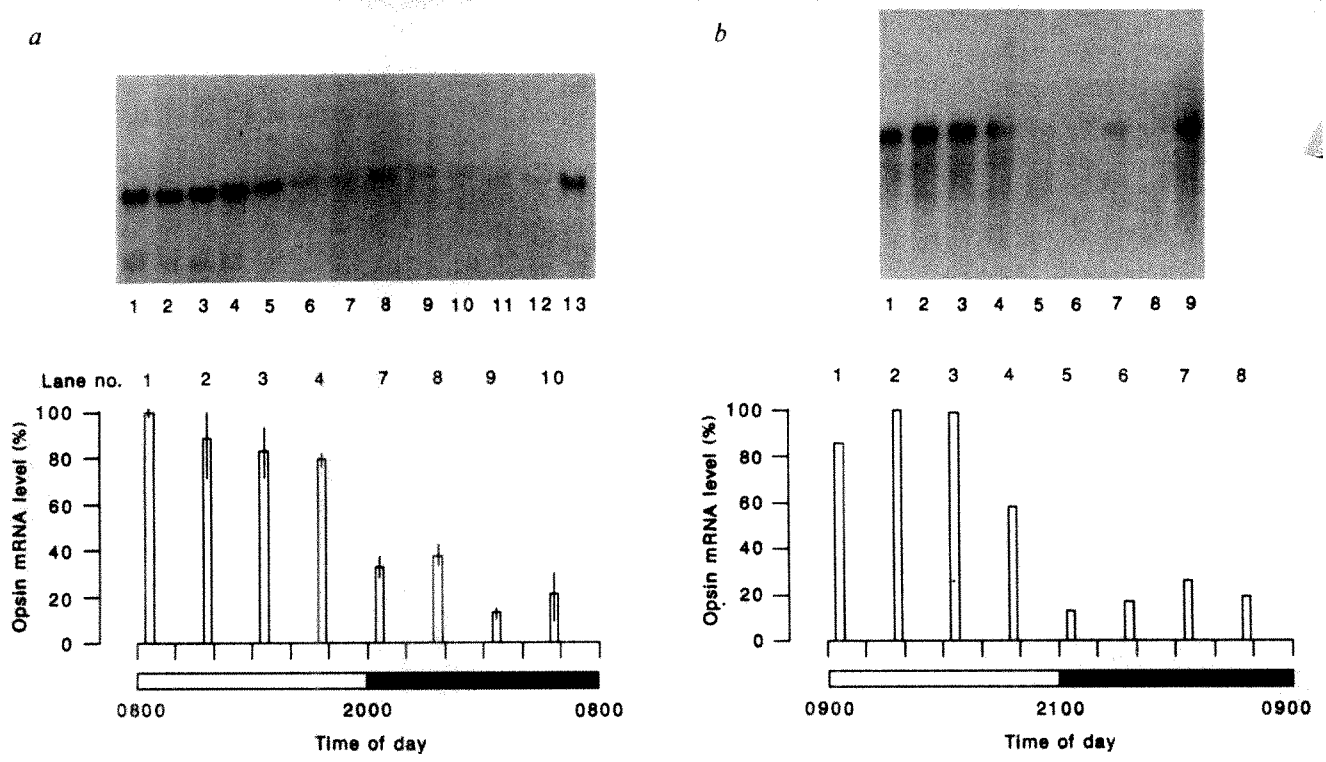


Fig. 2 Diurnal rhythm in the levels of retinal opsin mRNA in toad (*a*) and fish (*b*). The blot lanes contained total RNA pooled from two (toad) or six (fish) retinas of animals killed at intervals of three hours throughout a 12 h : 12 h light : dark diurnal cycle, beginning 0.5 h after lights on. In the autoradiogram of toad RNA (*a*) the identity and loading of each lane were: lane 1, 8:30 a,m,, 10 μg; lane 2, 11:30 a.m., 10 μg; lane 3, 2:30 p.m., 10 μg; lane 4, 5:30 p.m., 10 μg; lane 5, 5:30 p.m., 5 μg; lane 6, 5:30 p.m., 2.5 μg; lane 7, 8:30 p.m., 10 μg; lane 8, 11;30 p.m., 10 μg; lane 9, 11:30 p.m., 5 μg; lane 10, 11:30 p.m., 2.5 μg; lane 11, 2:30 a.m., 10 μg; lane 12, 5:30 a.m., 10 μg; lane 13, 8:30 a.m., 10 μg; sampled the following day. In the fish RNA blot (*b*) all the lanes were loaded with 10 μg: lane 1 at 9.30 a.m., with lanes 2–9 loaded with *samples collected* at three hourly intervals after that, so that lane 9 was loaded *with a sample collected* at 9.30 a.m. the following day. Densitometric analysis of the data and those of repeat experiments, is shown under the autoradiograms. The correspondence between RNA blot lanes and the histogram bars is shown by matching numbers. The density of each lane is expressed as a percentage of the greatest density measured in that autoradiogram. Bar height represents the average of 2–4 experiments and lines indicate the range of values measured. Comparison of RNA samples loaded in various amounts showed a typical error in loading of less than 10% (lanes 4–6).

Methods. All experiments were conducted in the winter, an important point as circadian rhythms and their entrainment can vary seasonally. Animals were adapted to a 12 h : 12 h light : dark cycle for at least six weeks. *Haplochromis* were bred from wild-caught stock and maintained at 28 °C. *Bufo* were obtained from commercial sources and maintainead at 25 °C in a constant temperature room. Illuminating light was spectrally matched to sunlight and its intensity was 3.1 W m^{-2} in the *Bufo* cage and 1.7 W m^{-2} at the water surface for *Haplochromis*. Autoradiograms were analysed with a laser densitometer (Ultrascan XL, LKB222-010, Bromma, Sweden).

that had experienced normal light cycles. Identical results were obtained for fish.

To investigate whether the increase in opsin mRNA during the daytime was synchronized with the light onset we measured the retinal mRNA level in toads killed at 2.5, 1.5 and 0.5 h before, and 0.5 h after light onset. At 2.5 h before lights-on, the opsin mRNA level was low, but it began to increase by 1.5 h before lights-on and was fully elevated 0.5 h before lights-on. Therefore the rise in opsin mRNA level is not activated by light onset but precedes it, suggesting that the levels of opsin mRNA are regulated by an endogenous clock.

We explored the relationship between light and the production of opsin mRNA by exposing toads and fish, entrained to a 12 : 12 in light : dark cycle, to 0.5 h of light at two different times during their normal dark period: 8 or 3 h before lights-on. In every case, the amount of opsin mRNA in animals exposed to light rose to day time levels (Fig. 3*d*). Thus, the mRNA level, although controlled by an endogenous rhythm can also respond directly to light.

We are now investigating whether the regulation we have observed in opsin mRNA is shared by other proteins in the rod. In higher vertebrates, diurnal variation in the levels of transducin mRNA[14,15] and opsin mRNA[15] has been observed, but whether these changes occur with an endogenous rhythm remains unexplored. Consistent with the rhythm of opsin mRNA level w describe, the amount of newly synthesized opsin in the frog ro inner segments can change by as much as 13-fold[16]. Becaus similar differences in amount were not observed in the oute segments[16], the variation in the amount of new opsin in th inner segments was presumed to reflect regulation of the siz of pools of opsin available for membrane synthesis. Our result suggest however, that changes in opsin content also reflec variation in the levels of its synthesis. Indeed, shortly afte light-onset there is fourfold increase in the amount of accumu lated opsin in the inner segment which declines only two hour after lights-off[17].

Although the kinetics of each step in outer segment renewa are not known for a single amphibian species, Fig. 4 is composite representation of the time course of events underlyin outer segment renewal from data obtained in *Rana*, *Xenopu* and *Bufo*. Disk membrane assembly[18] is delayed relative to th accumulation of rod opsin in the inner segment[17]. Opsi accumulation, in turn, lags behind the initial rise in cytoplasmi mRNA, which precedes light onset.

The selective advantages of diurnal variation in opsin mRN synthesis and disk membrane assembly are unknown. We specu late that the cone and rod renewal processes are out of phas with one another, rod opsin synthesis occurring mainly durin

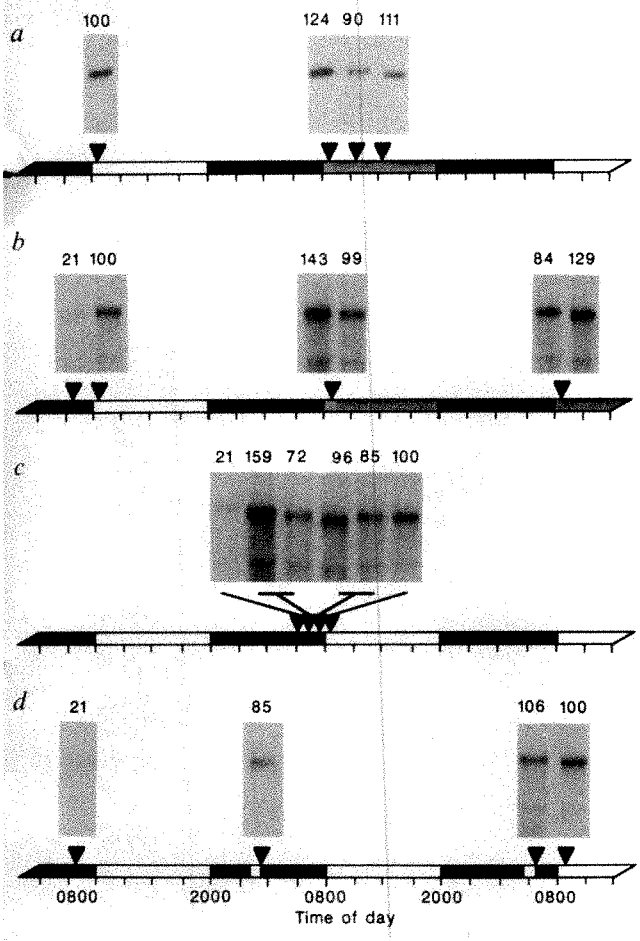


Fig. 3 Opsin mRNA levels are controlled by a circadian oscillator and are responsive to light. In each panel are autoradiograms from the same blot displayed to illustrate the experimental protocol. All lanes were loaded with 10 μg of toad retinal RNA. Band density relative to that measured 0.5 h after lights-on is expressed as a percentage and shown above each autoradiogram. *a*, The level of opsin mRNA rises in the absence of light during subjective daytime (indicated by the lightly shaded area). From the left, autoradiograms are from samples taken 0.5 h after lights on, and in darkness 0.5 h, 3.5 h and 6.5 h into subjective daytime. *b*, Opsin mRNA level is elevated at the time of subjective light onset after 24 or 48 h of continuous darkness. From the left, autoradiograms are from samples taken: in the dark, 2.5 h before lights turn on; in the light, 0.5 h after lights on; the next two in the dark, 0.5 h into the first subjective day; and the last two in the dark, 0.5 into the second subjective day. Samples from different animals are shown side by side to illustrate the range and reproducibility of the results. *c*, The increase in opsin mRNA level precedes light onset. From the left, sample identities are: in the dark, 2.5 h before light onset; the next two were in the dark, 1.5 h before light onset; the next two were in the dark, 0.5 h before light onset; the final one was in the light, 0.5 h after the light onset. *d*, Opsin mRNA levels rise in response to illumination during the night. Toads were exposed for 30 min to 2.9 W m^{-2} of continuous white light at two different times in the dark phase of their normal diurnal cycle. From the left, sample identities are: in the dark phase of the diurnal cycle (9 h of darkness) in the dark phase of the diurnal cycle (4 h of darkness) after 0.5 h illumination; in the dark phase of the diurnal cycle (9 h of darkness), after 0.5 h of illumination; in the light, 0.5 h after light onset.

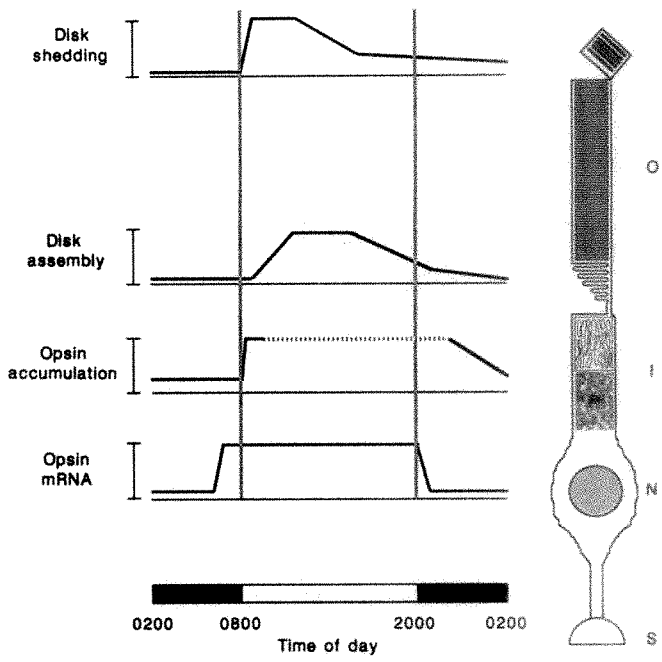


Fig. 4 Schematic illustration of the temporal and spatial relationships of events underlying rod disk assembly during a diurnal cycle based on data from three amphibia genuses maintained in a 12 h : 12 h light : dark cycle. A rod is sketched on the right with its major regions indicated: synapse (S), nuclear region (N), inner segment (I) and outer segment (O). The level of opsin mRNA in *Bufo* (compare with Fig. 2 and 3) increases one to two hours before light onset and remains high until shortly after dark onset. Opsin accumulation in the inner segment in *Rana*[17], begins at light onset and continues until two hours after dark onset. The rate of disk assembly in *Xenopus*[18] increases about one hour after light onset and decreases towards the end of the day. Disk shedding in *Xenopus*[18] and *Rana*[3] occurs once every four to five days when about 10% of the outer segment length is shed. Shedding begins at light onset and is completed within a few hours.

the day an cone opsin synthesis at night. If so, animals would have newly synthesized discs in place by dusk each day and rod photoreceptors would not be subject to structural changes at a time when vision is dominated by rod phototransduction.

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A novel eye in 'eyeless' shrimp from hydrothermal vents of the Mid-Atlantic Ridge

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***Rimicaris exoculata*[1] is a shrimp that swarms over high-temperature (350 °C) sulphide chimneys at Mid-Atlantic Ridge hydrothermal fields (3,600 m)[1–7]. This shrimp lacks an externally differentiated eye[1], having instead a pair of large organs within the cephalothorax immediately beneath the dorsal surface of the transparent carapace, connected by large nerve tracts to the supraesophageal ganglion. These organs contain a visual pigment with an absorption spectrum characteristic of rhodopsin. Ultrastructural evidence for degraded rhabdomeral material suggests the presence of photoreceptors. No image-forming optics are associated with the organs. We interpret these organs as being eyes adapted for detection of low-level illumination and suggest that they evolved in response to a source of radiation associated with the environment of hydrothermal vents.**

Instead of tubeworms, clams and mussels that are characteristic of vent sites on the East Pacific Rise, the fauna of Atlantic vents is dominated by shrimp. Several species coexist, but *Rimicaris exoculata* is by far the most abundant. As indicated by its specific name, *R. exoculata* lacks eyestalks and corneas[1]. Instead, the shrimp possesses a pair of highly reflective patches on the dorsal surface of the cephalothorax. Dissection shows these patches correspond to an anteriorly fused, bilateral pair of specialized organs (Fig. 1), interpreted here as being novel

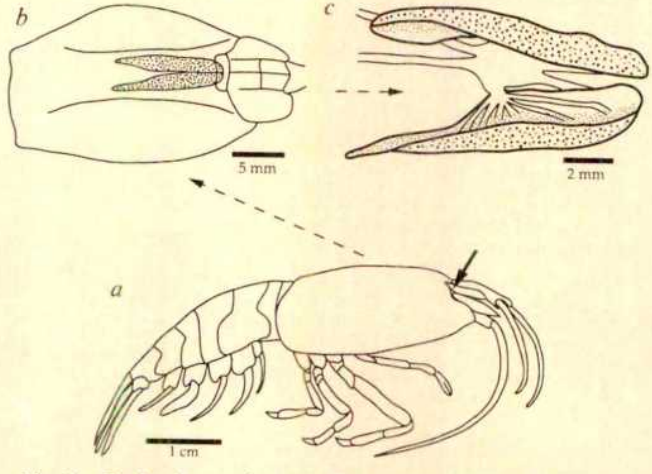


Fig. 1 *Rimicaris exoculata*. *a*, Lateral view. *R. exoculata* lacks eyestalks and conventional compound eyes. Solid arrow points to the location of eyes in the congeneric species *Rimicaris chacei*[1]. *b*, Oblique dorsal view showing the location of the novel visual organ (stippled area) underlying the thin transparent carapace. *c*, Dissection of the thoracic eye. The fused anterior tips have been separated along the midline to reveal the underlying connections to the supraesophageal ganglion.

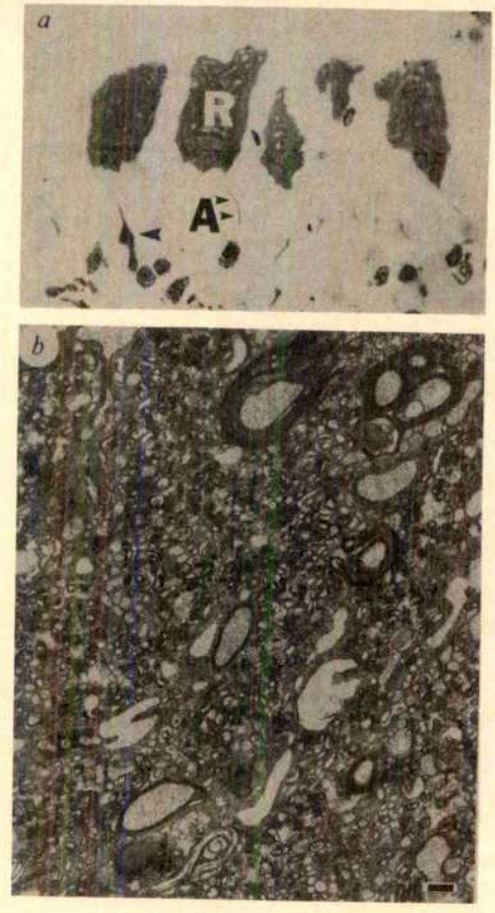


Fig. 2 Structure of photoreceptors of *Rimicaris exoculata*. *a*, Plastic 1-μm section stained with toluidine blue and cut normal to the eye surface. The rhabdomeral segment (R) is filled with a large elaboration of rhabdomere. The arhabdomeral segment (A) is severely attenuated (right arrowheads). The left arrowhead shows a photoreceptor nucleus. No other cellular elements are present. *b*, Electron micrograph of a region of rhabdomeral segment cytoplasm. The vesicles and whorls of membrane probably represent degraded microvilli from the rhabdom. The bar in *b* represents 13 μm in *a* and 0.5 μm in *b*.

eyes. The dorsal quarter of each eye is a compact zone of textur tissue which gives way ventrally to a fibrous web. Fibres of th web coalesce ventromedially into a nerve trunk that enters t fused supraesophageal ganglion in the same position as t optic nerve of other decapods[8]. Examination of 15-μm seri sections of paraffin-embedded tissue stained with haematoxyl and eosin reveals that each fan is composed of ommatidia-lil clusters of photoreceptors (5–7 cells per cluster; average, 6 There is no evidence for a lens or dioptric apparatus associate with each 'ommatidium'. Long axes of these 'ommatidia' a oriented perpendicularly to the carapace along the dorsal surfa of the organ. Each shrimp has about 9×10^3 receptor cells.

The distal portion of each photoreceptor is a cylindric rhabdomeral segment filled with microvillar membrane; th proximal portion is a highly attenuated arhabdomeral segme that contains the nucleus (Fig. 2*a*, left arrowhead). The ultr structure of the rhabdomeral segment (Fig. 2*b*) is almost ce tainly degraded from the living state[9–16] because the anima were fixed only after exposure to light at the ocean's surfac Nonetheless, the arrays of membranous vesicles and whorls ar suggestive of broken-down microvillar rhabdom as found i other invertebrate photoreceptors[12–16]. The reflective propert of the organ suggests a tapetal mechanism for increased ligh absorption by the receptors. As the reflective property is n

preserved in frozen or fixed specimens, we cannot determine the chemical nature of the tapetum, nor the extent to which it contributes to image-formation, if at all. Reflective cells[10-12] and extremely attenuated arhabdomeral segments[12] have been demonstrated in other crustaceans adapted to low ambient light levels. Thus, morphological evidence indicates that the unusual organs in *Rimicaris* are modified compound eyes, specialized for high sensitivity by extreme proliferation of rhabdomeral membrane, deletion of a dioptric apparatus, and presence of a tapetum.

Any organ specialized for photoreception should contain a rhodopsin-like protein. We performed rhodopsin assays on the available light-adapted specimens. Rhabdomeral membranes were partially purified by either isopycnic centrifugation on sucrose gradients[17] or differential centrifugation, and pigment was extracted from the membranes with digitonin. In the presence of hydroxylamine (NH_2OH), light bleaches a shrimp pigment that maximally absorbs at 500 nm and produces a product that maximally absorbs at 366 nm (Fig. 3). The difference spectrum closely resembles that of vertebrate rhodopsins. The location, relative magnitude and shape of the absorption bands indicate that the shrimp pigment is rhodopsin and that the product of the light-induced reaction is all-*trans*-retinaloxime. Formation of all-*trans*-retinaloxime, whose absorption peak is at 365-367 nm (ref. 17), indicates that before irradiation the chromophore is retinal, and is in a *cis*-isomer configuration[17]. Formation of retinaloxime also indicates that metarhodopsin is unstable to hydroxylamine under our experimental conditions. In this respect, pigment from *R. exoculata* resembles visual pigments of pelagic euphausiids[18].

Assuming the usual extinction coefficient for rhodopsin[17], 25 pmol pigment was extracted from each organ (50 pmol per shrimp) in the experiment shown in Fig. 3. This is a lower limit, because it ignores the fraction of pigment in the metarhodopsin state and because the limited amount of shrimp tissue prevented us from optimizing experimental conditions. Compared with crayfish[19] and horseshoe crabs[20], the eyes of *R. exoculata* contain at least 2-7 times more visual pigment.

Rhodopsin in *R. exoculata* absorbs maximally at a slightly longer wavelength than has been reported for other deep-sea-dwelling crustacea. For example, visual pigments of two species of pelagic euphasiids peak at 485-488 nm in digitonin extracts[18] and in mesopelagic decapods, λ_{max} varies from 485 to 495 nm by microspectrophotometry[21]. Some of these shrimps were also studied with electrophysiological techniques and showed maximal spectral sensitivity at about 500 nm (ref. 22).

Presence of a visual pigment supports morphological evidence that the thoracic organ is an eye; the unusual nature of this eye suggests it has a novel function. In the typical deep sea at depths of 3,600 m, bioluminescence is the only known source of ambient light. Macroorganisms produce flashes of relatively high-intensity light[23] with emission maxima clustered at around 460-490 nm[22]. In general, the λ_{max} of a visual pigment closely matches the spectral distribution of the ambient light. So it is tempting to relate the visual pigment, with its λ_{max} of 500 nm, to detection of bioluminescent phenomena by the shrimp. But then why should a new sensory organ evolve in place of a normal crustacean eye which is an adequate detector of visible light for most pelagic decapods?

The dominant physical features of the shrimps' environment are plumes of water at 350 °C. Detection of plumes could attract shrimp to feeding areas[7] and deter them from plunging into water hot enough to cook them. In a medium that does not transmit infrared radiation and in an environment of very steep thermal gradients (>100 °C per cm), detection of plumes with temperature-sensitive receptors is probably inadequate. Can *Rimicaris* 'see' plumes of hot water? Visual detection might be possible if the plumes emit light. Sources of light could be chemical (as in oxidation-reduction reactions or thermoluminescence) or physical (for example, Cherenkov radiation from radioisotopes or thermal black-body radiation). Of these possibilities, only thermal radiation from 350 °C water is a certain source of visible light.

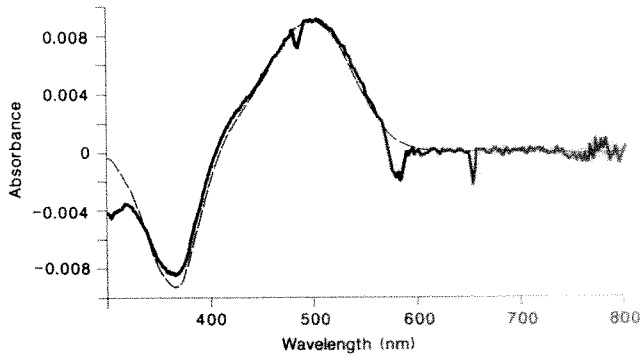


Fig. 3 Bleaching difference spectrum of visual pigment in *Rimicaris exoculata* (solid trace), obtained by subtraction of spectra measured before and after bleaching. Absorption due to reactants is positive and to products is negative. Bleaching destroys a shrimp pigment that maximally absorbs at 500 nm, typical of 'classical' rhodopsin, and creates a new pigment with a λ_{max} at 367 nm, which corresponds to retinaloxime. The same features are also seen in the difference spectrum of frog rhodopsin (dashed line) normalized to maximum absorbance at 500 nm. The slight mismatch in amplitudes of retinaloxime products may be attributed to a slightly greater (1.08 times) extinction coefficient for shrimp rhodopsin. Spikes at 660, 580 and 490 nm are instrumentation artefacts that regularly appear at these wavelengths.

Methods. All experiments were performed under dim red light with light-adapted animals. Organs of six thawed shrimps were dissected and homogenized in a solution of artificial cytoplasmic medium (80 mM NaCl, 335 mM Kisethionate, 200 mM sucrose, 4 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES titrated to *p*H 7.4 with NaOH)[26] including protease inhibitors (0.5 mM PMSF, 7 μg ml^{-1} of pepstatin, 50 μg ml^{-1} of leupeptin). The homogenized suspension was centrifuged for 30 min at 10,000*g*. The resulting pellet was resuspended in a hypotonic medium (10-fold dilution of the artificial cytoplasmic medium) and was centrifuged for 30 min at 51,000*g*. The final pellet was solubilized for 3 h at room temperature with 2% digitonin in amphibian saline solution (115 mM NaCl, 2.5 mM KCl, 1.5 mM $MgSO_4$, 10 mM HEPES titrated to *p*H 7.4 with 4 mM NaOH). Unsolubilized material was removed by centrifugation (2 min at 13,000*g*). The clear supernatant was used for subsequent analysis on a diode-array spectrophotometer (HP 8452, Hewlett-Packard). After the initial spectrum was recorded, 45 mM NH_2OH was added to the solution in the cuvette to destroy any retinochrome[27] present. Subsequently, the cuvette contents were exposed for 5 min to a light source that bleached ~95% of the rhodopsin. For about a 10-min interval both before and after bleaching, the time course of any dark reaction was monitored because the initial tissue homogenate contained relatively large concentrations of water-soluble pigments which could interfere with spectral analysis. In this experiment, the contribution of the dark-reaction was negligible and so no correction was made to the recorded bleaching difference spectrum. We obtained the same bleaching difference spectrum with rhabdoms that were purified by sucrose flotation[17]. We specifically searched the rhodopsin extract for the presence of membrane pigments that absorb in the red end of the visible spectrum. Our spectral analysis extended to 900 nm, but no pigment was detected that was preferentially sensitive to the longer wavelengths. The entire protocol from homogenization to extraction was also performed on control tissue derived from the abdomen of the shrimp, where no rhodopsin was detected.

Photons within the visible spectrum represent a very small fraction of the total photon flux emitted by a black body at 350 °C. But they may be sufficient to exceed the presumed threshold in shrimp[24]. If so, effective photon absorption by *R. exoculata* would be maximal at about 600 nm, given the spectral sensitivity of its rhodopsin. As a test of visibility of hot objects

with a 500 nm pigment, we demonstrated that dark-adapted humans can see a laboratory hot plate heated to 375 °C. *In situ* measurements of ambient light levels and the spectral characteristics and attenuation at hydrothermal vents are only just beginning[25].

We have demonstrated that the vent shrimp, *Rimicaris exoculata*, previously thought to be eyeless, has a thoracic eye that is well adapted for detection of very dim light. Thermal radiation from high-temperature plumes at black smoker chimneys could provide the light sensed by the shrimp. The role of the thoracic eye as a visual organ, however, will remain unresolved until more is known about its physiology and its unusual photic environment.

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The visibility of 350 °C black-body radiation by the shrimp *Rimicaris exoculata* and man

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The eye of the 'eyeless' shrimp *Rimicaris exoculata* is unusual in having no image-forming optics and a high concentration of rhodopsin[1]. The shrimps swarm around 350 °C hydrothermal 'black smoker' vents in the Mid-Atlantic Ridge[2]. There is no other known source of visible light in the shrimp's environment. The spectral sensitivity of rhodopsin is well matched to typical spectra of bioluminescence of organisms found at lesser depths, but other animals detect such emissions without the unusual features of the *R. exoculata* eye[1]. These two features are most easily understood as an adaptation for the detection of extremely faint sources o light. Physical calculations presented here indicate that the shrim could see the black-body radiation of the 350 °C vents, even thoug these sources are practically invisible to the human eye. This woul be useful to the shrimp as it feeds on sulphide-loving bacteria ver near to the vents[3] but must avoid the lethal 350 °C vents themselve

In the absence of behavioural evidence, no calculation ca prove that the shrimp sees anything. Therefore we take th opposite approach, assuming reasonable values for the fe unknown parameters, to show that the known physical con straints do not preclude rhodopsin-mediated detection of 350 °C black-body radiation by *R. exoculata*. We begin with the spectra of black-body emission and rhodopsin photosensitivity, and th amount of rhodopsin (50 pmol) in each shrimp eye[1]. We assum only that the black smoker is a black-body radiator, that ther is no intervening absorption over visible wavelengths by th deep sea water or the carapace of the eye, that a reflective laye at the back of the eye (which is apparent in photographs of liv shrimp[1]) reflects all light incident upon it, that the dark ligh (spontaneous isomerizations) in the shrimp eye is comparabl to that in other rhodopsin-based eyes, and that the shrimp' nervous system allows it to count (integrate) isomerizations from the entire eye.

A related question is whether this radiation would be visibl to man. Partially dark-adapted operators of the Alvin submarine parked next to the vent, were unable to see anything without artificial illumination (C. L. Van Dover, J. R. Delaney, L. M. Smith, J. R. Cann and D. B. Foster, manuscript in preparation and ref. 4). They did manage, however, to image the hydrothermal vent itself by a long-exposure photograph on a CCD (charge-coupled device) camera.

The spectral photon radiance of blackbody radiation is:

$$N_{e\lambda}(T,\lambda) = 2c\lambda^{-4}(e^{hc/kT\lambda} - 1)^{-1}\ \mathrm{sr}^{-1}, \qquad (1)$$

where T is temperature, λ wavelength, h Planck's constant, c the speed of light, k Boltzmann's constant, and sr is a steradian[5]. The dashed curve in Fig. 1*a* shows this spectrum for a 350 °C black body, relative to its maximum at wavelength 5,889 nm (wavenumber 0.17 μm^{-1}), well outside what is usually considered the visible range. The relative quantum efficiency $\alpha(\lambda)$ of rhodopsin is shown as a dotted curve, peaking at 500 nm (wavenumber 2 μm^{-1})[6]. Absorption is proportional to the product of radiation and quantum efficiency, and is shown as the dotted-and-dashed curve in Fig. 1*a*, relative to its maximum at 588 nm. Note that although the radiation and efficiency curves have very different peaks, at opposite ends of Fig. 1*a*, their tails overlap and have nearly complementary slopes, resulting in a nontrivial absorption peaking at an intermediate wavelength within the 'visible' range. We have not taken into account the transmission of the intervening water or optics—the carapace over the eye—because we do not have good *in situ* measurements over the relevant wavelengths. Distilled water is, however, transparent over the relevant range (200–1,500 nm) and the transmission outside this range has a negligible effect on the calculated total absorption at 350 °C.

Taking into account the radiation and efficiency, the equivalent number of 500 nm photons $s^{-1}\ m^{-2}\ sr^{-1}$ is:

$$N_e(T) = \int_0^\infty \alpha(\lambda) N_{e\lambda}(T,\lambda)\, d\lambda \qquad (2)$$

Assuming the eye is directed toward the 350 °C vent, that the vent is a sphere whose radius subtends an angle ω at the eye, and that the light makes two passes through the rhodopsin (before and after reflection at the back of the eye), the number of photons absorbed is:

$$N_a(T) = 2.9 \times 10^{-6}\ \mathrm{m^2\ sr}\ \sin^2(\omega) N_e(T) \qquad (3)$$

This is plotted in Fig. 1*b* as a function of temperature, assuming that the vent is sufficiently close to fill the shrimp's visual field,

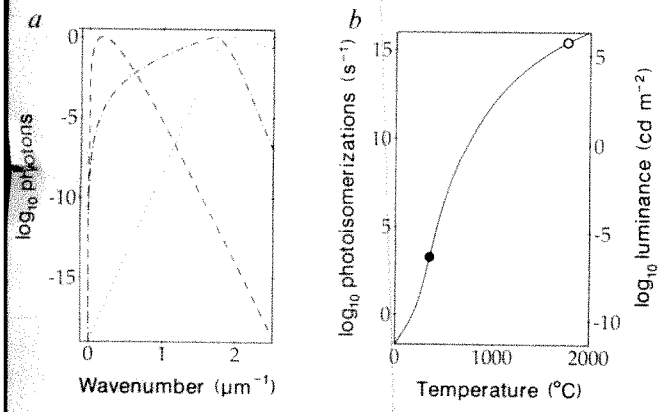


Fig. 1 *a*, The dashed line is the spectral photon radiance $N_{e\lambda}(T, \lambda)$ of 350 °C black-body radiation as a function of wavenumber, $1/\lambda$. The dotted line is the quantum efficiency $\alpha(\lambda)$ of rhodopsin. The dotted-and-dashed line is the product $N_{e\lambda}(T, \lambda)\alpha(\lambda)$. All three plots are relative to their maximum values. *b*, The number of photoisomerizations per second $N_a(T)$ in the shrimp eye (left-hand scale, assuming $\omega = \pi/2$) and the luminance $L'(T)$ (right-hand scale) as a function of the temperature of the black-body radiator. At 350 °C (●) the shrimp eye absorbs 1,800 photons per second under optimal conditions and the luminance is 0.43×10^{-6} cd m^{-2}. The open symbol (○) is explained below.

Methods. Taking into account the angular subtense ω of the target and the area A of the eye, the 500-nm-equivalent photon flux incident on the eye is $N(T) = \pi \sin^2(\omega)\,\mathrm{sr} \times A N_e(T)$. The total number of photons that are absorbed and isomerize rhodopsin in two passes (before and after reflection by the reflective layer) will be $N_a(T) = (1 - t_r^2)\,\gamma N(T)$, where γ is the quantum efficiency for isomerization and t_r is the transmission of the rhodopsin, which is given by $t_r = 10^{-\varepsilon C d}$, where ε is the molar absorbance at 500 nm, C is the concentration, and d is the path length. As the transmission is nearly 1, we neglect self-screening and make the approximation $(1 - t_r^2)\gamma = (1 - 10^{-2\varepsilon C d})\gamma \approx 2\ln(10)\varepsilon C d\gamma$. The product $\varepsilon\gamma$ is called photosensitivity and is approximately 4.06×10^4 l cm^{-1} mol^{-1} at 500 nm for rhodopsin *in vivo*[9]. Thus $N_a(T) = (2\ln(10)\varepsilon\gamma C d)\,N(T)$, which yields equation 3. The area A and thickness d of the rhodopsin layer in the eye cancel out; all that matters is the total amount of rhodopsin. We estimated the efficiency $\alpha(\lambda)$ of the rhodopsin by a Dartnall nomogram peaking at 500 nm, using Dartnall's long-wavelength extrapolation of that nomogram[6], that is, an asymptotic gradient $\Delta\log[\alpha(\lambda)]/\Delta(1/\lambda)$ of 1.05×10^{-3} cm. As a check, note that at 2,042K = 1768.85 °C (the open symbol, ○) the luminance is approximately 60,000 cd m^{-2}, in agreement with the 1967 definition of the candela[5]. The scotopic quantum efficiency of a standard human observer $W'_\lambda(\lambda)$ is approximately equal to the 500-nm rhodopsin quantum efficiency curve $\alpha(\lambda)$, except at short wavelengths (<500 nm), where lens absorption reduces efficiency, and at very long wavelengths (>780 nm) where $W'_\lambda(\lambda)$ is zero[5]. Substituting the standard scotopic relative quantum efficiency function $W'_\lambda(\lambda)$ for $\alpha(\lambda)$ in the definition of $N_{e\lambda}(T, \lambda)$ would have a negligible effect on the computed luminance at temperatures in the range 500 to 2,000 °C, but would have an ever-increasing effect at temperatures below 500 °C. At 350 °C the scotopic luminance is 0.43 10^{-6} cd m^{-2} assuming $\alpha(\lambda)$ but would be only 0.16×10^{-6} cd m^{-2} if we were to assume $W'_\lambda(\lambda)$ instead.

that is $\omega = \pi/2$. At 350 °C the photoisomerization rate is 1,800 s^{-1} (● in Fig. 1*b*).

We assume that isomerizations caused by photon absorption are indistinguishable from thermal isomerizations. Using the Baylor estimate[7] of the rate of thermal isomerization of rhodopsin (in monkey rods), 50 pmol of rhodopsin present in the shrimp eye will yield approximately 1,600 thermal isomerizations per second. To detect the 350 °C vent, the shrimp would have to discriminate a signal-plus-noise isomerization rate of 1,800 + 1,600 = 3,400 s^{-1} from a noise-alone rate of 1,600 s^{-1}. A brief integration time of only 20 ms would yield a decision variable with a signal-to-noise ratio of 6.4 (the mean signal divided by the standard deviation of the noise). This would allow the shrimp to detect correctly the lethal hot water 99.9% of the time, and to mistake ambient for hot water only 2% of the time. The photon flux and signal-to-noise ratio will, however, fall quickly if the shrimp is farther from the vent. Assuming a long integration time of 1 s, then for the shrimp to obtain a minimum useful signal-to-noise ratio of 1 (correctly detect hot water 50% of the time and mistake ambient for hot 16% of the time) the 350 °C vent must subtend at least $2\omega = 2.5°$. Thus a 10 cm wide vent could be detected from as far away as 2.3 m.

To assess the visibility of the vent by man we estimate its scotopic luminance:

$$L'(T) = \frac{1{,}700\ \mathrm{lm}}{\mathrm{W}} \frac{hc}{507\ \mathrm{nm}} \frac{N_e(T)}{\alpha(507\ \mathrm{nm})} \qquad (4)$$

where lm is a lumen. This is plotted in Fig. 1*b* over the temperature range 0 to 2,000 °C, using the right-hand vertical scale. At 350 °C (●) the luminance is 0.43×10^{-6} cd m^{-2}, which is slightly below the average threshold of 0.75×10^{-6} cd m^{-2} found by Pirenne[8] for seeing a large long-duration uniform disk by fully dark-adapted observers with natural pupils and unrestricted eye movements. Thus the operators of the Alvin submarine failed to see the vents by naked eye[4] because the vents were just below the threshold of visibility.

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Segment-specific expression of a zinc-finger gene in the developing nervous system of the mouse

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The process of segmentation, in which repeated homologous structures are generated along the anterior–posterior axis of the embryo is a widespread mechanism in animal development. In vertebrates, segmentation is most apparent in the somites and the peripheral nervous system[1,2], but the existence of repetitive bulges, termed neuromeres, in the early neural epithelium of vertebrates suggests that the CNS may also be segmented[3–9]. Consistent with this, cranial ganglia[8] and certain neurons[8,10] are associated with specific hindbrain neuromeres. Here, we report that *Krox*-20, a zinc-finger gene, is expressed in two alternate neuromeres in the mouse early hindbrain. This pattern subsequently decays and *Krox*-20 is transiently expressed in specific hindbrain nuclei. In addition, *Krox*-20 is expressed in early neural crest cells, and then in the neural crest-derived boundary caps, glial components of the cranial and spinal ganglia. The demonstration that neuromeres are domains of gene expression provides molecular evidence for the segmentation of the CNS.

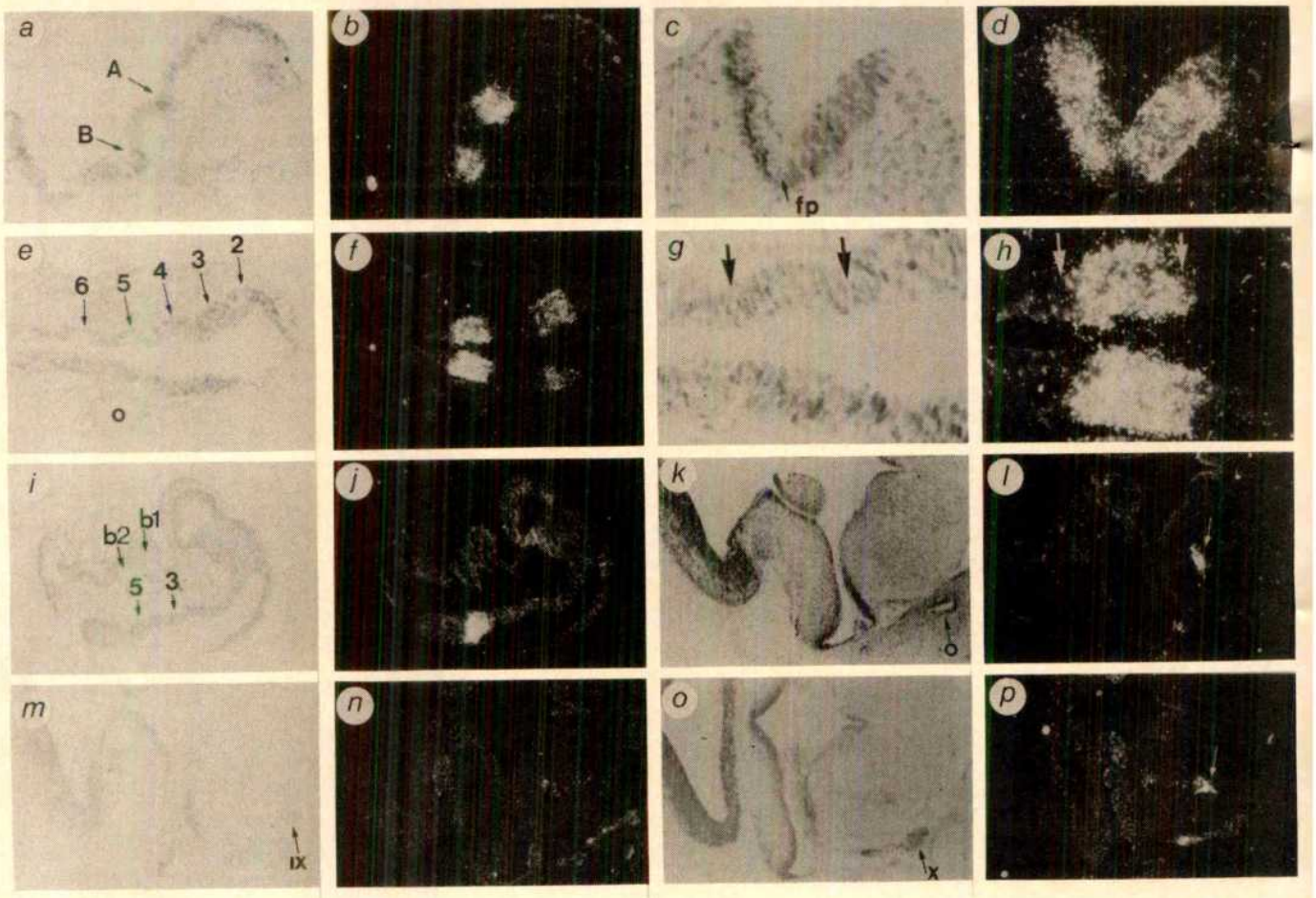


Fig. 1 *In situ* hybridization of ^{35}S-labelled antisense RNA probes for *Krox*-20 to sections of mouse embryos. *a*, *b*, Longitudinal section of 8.5-day-old embryo. *c*, *d*, Transverse section through proneuromere B of 8.5-day-old embryo. *e*, *f*, Coronal section through hindbrain of 9.5-day-old embryo. *g*, *h*, Higher magnification view of neuromere 5 in *e*, *f*; arrows indicate neuromere boundaries. *i*, *j*, Parasagittal longitudinal section of 10-day-old embryo. *k*–*p*, transverse sections through increasing caudal regions of the floor of the hindbrain of a 14.5-day-old embryo. *a*, *c*, *e*, *g*, *i*, *k*, *m* and *o* are brightfield photographs; *b*, *d*, *f*, *h*, *j*, *l*, *n* and *p* are the corresponding darkfield photographs. Embryos are staged according to the developmental ages described by Theiler[31]. Proneuromeres are designated A and B, and neuromeres are numbered 2, 3, 4, 5, 6 (see text for details). fp, floor plate; o, otocyst; b1, 1st branchial arch; b2, 2nd branchial arch; IX, glossopharyngeal ganglion; X, vagal ganglion.

Methods. *In situ* hybridization was performed as described[32]. The probe corresponded to *Krox*-20 3′ untranslated sequences (residues 2,417–2,834 of the cDNA, see ref. 11). Identical expression patterns were observed using a 5′ probe (residues 523–883 of the cDNA), although this gave a higher non-specific background (data not shown). No signals above background were observed in sense-strand control hybridizations (data not shown).

Krox-20 was originally identified as a serum-responsive gene in mouse NIH 3T3 fibroblasts, where its transcription is rapidly and transiently activated by the addition of serum or purified growth factors to quiescent cells[11,12]. *Krox*-20 encodes a protein with three zinc fingers similar to those of the transcription factor Sp1[11]. It therefore seems likely that *Krox*-20 encodes a transcription factor that has a role in early events in the resumption of growth in fibroblasts. A 3.2 kilobase (kb) *Krox*-20 transcript is detected in the thymus, spleen and testis of adult mice[11], and also in developing embryos (data not shown). We used *Krox*-20 probes for *in situ* hybridization analysis to examine the spatial and temporal pattern of expression of *Krox*-20 during mouse development.

Krox-20 transcripts were not detected in early gastrulation stage (7.5-day-old) mouse embryos (data not shown), but at 8.5 days they were found in two regions of the neuroepithelium of the prospective hindbrain (Fig. 1*a*, *b*). No other sites of *Krox*-20 expression were detected in serial longitudinal and transverse sections of the CNS. The regions of *Krox*-20 expression are in the vicinity of two invaginations of the neuroepithelium termed proneuromeres A and B[8]. Proneuromere B coincides with the centre of a site of *Krox*-20 expression, but proneuromere A does not, and analysis of serial sections revealed no simple spatial relationship between proneuromeres and sites of *Krox*-20 expression. At this stage, these sites of *Krox*-20 expression are 8–10 cells in length. In transverse sections through sites of expression, *Krox*-20 transcripts were detected throughout the neuroepithelium, except for the floor plate (Fig. 1*c*, *d*). By 9.5 days, seven neuromeres had formed in the hindbrain, and longitudinal (data not shown) and coronal sections (Fig. 1*e*, *f*) revealed that *Krox*-20 transcripts are restricted to two alternate neuromeres: neuromeres 3 and 5 of the hindbrain (neuromere 5 flanks the anterior end of the otocyst[8]). The boundaries of *Krox*-20 expression coincide with the sulci separating neuromeres (Fig. 1*g*, *h*). At 10 days of development, *Krox*-20 expression has been down-regulated in neuromere 3, but persists in neuromere 5 (Fig. 1*i*, *j*), and by 10.5 days expression is barely detectable in the hindbrain (data not shown). The down-regulation of expression in neuromere 3 before neuromere 5 may reflect the anterior–posterior direction

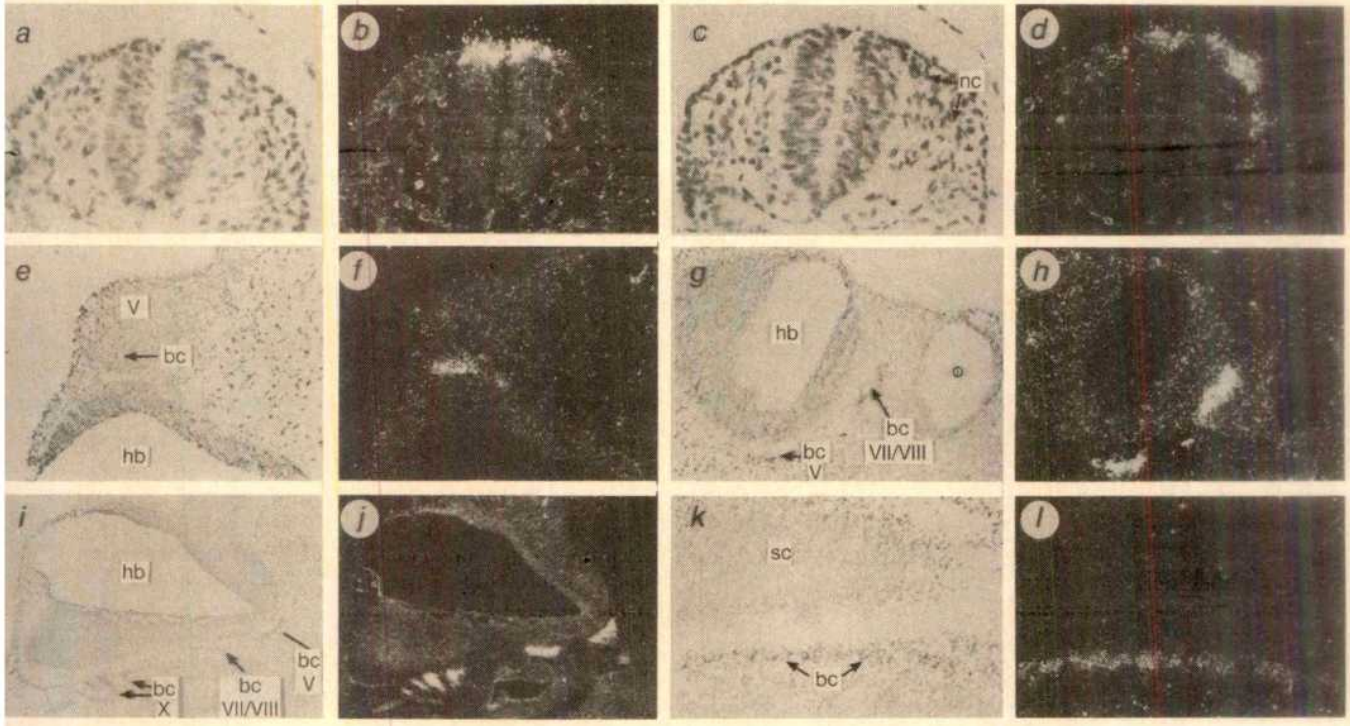


Fig. 2 Expression of *Krox*-20 in the developing peripheral nervous system. The pattern of *Krox*-20 expression was determined by *in situ* hybridization of radiolabelled probes to some embryo sections. *a, b*, Transverse section of 8.5-day-old embryo through region immediately caudal to proneuromere B (see Fig. 1*c, d*). *c, d*, Transverse section of 8.5-day-old embryo caudal to that shown in *a, b*. *e, f*, Transverse section through trigeminal ganglion of 10.5-day-old embryo. *g, h*, Parasagittal longitudinal section through hindbrain of 10.5-day-old embryo. *i, j*, Parasagittal longitudinal section through hindbrain of 12.5-day-old embryo. *k, l*, Longitudinal section through spinal cord of 14.5-day-old embryo. Arrows indicate sites of *Krox*-20 expression. Boundary caps (bc) of trigeminal (V), facial/vestibular (VII/VIII), vagal (X) and dorsal root ganglia are labelled. nc, Neural crest; hb, hindbrain; o, otocyst; sc, spinal cord. Methods were as in Fig. 1 legend.

of CNS maturation. *Krox*-20 expression was not detected in the CNS at 12.5 days of development, but at 14.5 days there was expression in two columns of cells in the hindbrain. Serial transverse sections reveal *Krox*-20 expression in a dorso-lateral group of cells (Fig. 1*k, l*) that extend from the level of the vestibular ganglion to the glossopharyngeal ganglion. Only low levels of *Krox*-20 transcripts are detected immediately caudal to the latter ganglion (Fig. 1*m, n*), but a more ventro-medial column (Fig. 1*o, p*) of *Krox*-20-expressing cells is detected from the level of the vagal ganglion to the caudal end of the hindbrain. The identity of these sites of expression is unclear because of the rapid changes in the formation and migration of hindbrain nuclei at this time. *Krox*-20 expression was not detected in the CNS of 16.5-day-old embros; this was not due to poor retention of RNA as other probes can detect similar levels of transcripts in the hindbrain of 14.5-day-old and 16.5-day-old embryos. Thus, *Krox*-20 seems to be transiently expressed in specific nuclei of the hindbrain.

Analysis of serial transverse sections through 8.5-day-old embryos revealed *Krox*-20 transcripts in neural crest cells as well. In regions immediately caudal to proneuromere B (see Fig. 1 *a–d*), *Krox*-20 transcripts are detected in the dorsal neural epithelium (Fig. 2*a, b*), where neural crest cells originate, and, in more caudal sections, early migratory neural crest cells express *Krox*-20 (Fig. 2*c, d*). At 10.5 days of development, *Krox*-20 expression was detected in boundary cap cells of the trigeminal and vestibular/facial ganglia (Fig. 2*e–h*). Boundary caps are comprised of neural crest-derived glial cells[13] that are located adjacent to the neuroepithelium at the sites of its penetration by sensory afferent and motor efferent axons[14]. By 12.5 days, *Krox*-20 expression was detected in the boundary caps of all the cranial ganglia (Fig. 2*i, j* and data not shown) and the sensory and motor roots of the dorsal root ganglia of the spinal cord (Fig. 2*k, l*, and data not shown). The detection of *Krox*-20 transcripts in certain early neural crest cells suggests that these precursors of the boundary cap cells, whose differentiation has been initiated during, or before, neural crest migration. Although much of the neural crest seems uncommitted in its fate at early stages of migration[15], there is evidence for early determination of some neural crest populations[16,17]. In view of the association of *Krox*-20 gene transcription with the G_0 to G_1 transition in fibroblasts[11,12], expression of this gene could be associated with the proliferation of boundary cap cells[14]. *Krox*-20 expression does not, however, correlate with cell division in the neuromeres, and later occurs in post-mitotic neurons.

Despite much debate about the possible developmental significance of neuromeres[3–9], there was no direct evidence for segmentation in the hindbrain of higher vertebrates. Our observation that certain neuromeres are domains of *Krox*-20 expression provides molecular evidence that neuromeres are segments. Localized *Krox*-20 expression precedes the formation of neuromeres, suggesting that segmentation may occur before it is manifested at the morphological level. The absence of a correlation between *Krox*-20 expression and proneuromeres is consistent with the lack of correspondence between these early-forming sulci and neuromeres in the rodent embryo[9]. Recent studies of neuronal development in the chick hindbrain have shown that it occurs in a segmental pattern in hindbrain neuromeres (rhombomeres) 2–6[18]. Motor neuron formation is first initiated in neuromeres 2, 4 and 6, and then in neuromeres 3 and 5, yielding an alternating pattern of cellular development. The alternating pattern of *Krox*-20 expression in the segmented region of the hindbrain is intriguing, in view of the similar expression pattern of pair-rule segmentation genes in the

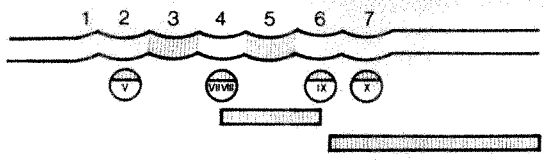


Fig. 3 Summary of sites of *Krox*-20 expression, showing the spatial relationship between the various sites of *Krox*-20 expression. In the upper part, the neuromeres (1-7) are shown; segmentation at the level of cellular development occurs within neuromeres 2-6 (ref. 18), and *Krox*-20 expression occurs in alternate neuromeres within this domain. Below are aligned representations of the cranial ganglia and boundary caps (V-X). Cranial ganglia caudal to the neuromeres are not indicated. The lower part of the figure shows the relative position of the nuclei that express *Krox*-20 at 14.5 days of development. The sites of *Krox*-20 expression are indicated by shading.

Drosophila melanogaster embryo[19,20]. It is possible, however, that *Krox*-20 expression may be associated with phenotypic characteristics unique to neuromeres 3 and 5. For example, motor neuron formation is delayed in these neuromeres, and they are the only definitive neuromeres not adjacent to cranial ganglia (Fig. 3). There is no obvious relationship between the transient expression of *Krox*-20 in neuromeres and, later on, in specific hindbrain nuclei; the rostral column of cells expressing *Krox*-20 overlaps with the hindbrain region derived from neuromere 5, but the more caudal nucleus has no such overlap (Fig. 3). Thus, it seems likely that *Krox*-20 acts in distinctive cell populations at different stages of hindbrain development. There are other examples of this behaviour for genes with early segment-restricted expression, as many segmentation and homoeotic genes in *Drosophila* are subsequently expressed in certain neurons in all segments[21].

Our data suggest that *Krox*-20, as a putative transcription factor, could be part of a regulatory network coupled to hindbrain segmentation. The expression of mouse homoeobox (*Hox*) genes in restricted anterior-posterior domains of the neural tube suggests that they may specify position along the body axis[22,23]. Many of these genes have anterior limits of expression in the hindbrain, although a possible relationship with neuromeres has been observed only for *Hox*-1.5[24]. It is therefore pertinent to investigate whether *Krox*-20 interacts with *Hox* genes or with other members of the mouse zinc-finger gene family[25-26]. One such gene, *mKr2*, is expressed throughout the developing CNS and peripheral nervous system[27], and the mouse genome contains at least one other zinc-finger gene closely related to *Krox*-20[28-30]. It will be interesting to determine whether other members of this group are expressed in the developing nervous system and, in particular, whether any have neuromere-restricted domains of expression.

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Transgenic mice overexpressing the mouse homoeobox-containing gene *Hox-1.4* exhibit abnormal gut development

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The mouse homoeobox-containing genes exhibit temporally an spatially specific patterns of expression in embryonic and adul tissues and are thought to be important in regulation of develop ment and cellular differentiation, perhaps by mechanism analogous to homoeotic genes in *Drosophila melanogaster*[1-4] There has been no direct demonstration that expression of thes mammalian genes can affect developmental processes, howeve *Hox-1.4*, like other mouse homoeobox-containing genes, has bee shown to be expressed in specific regions of the mid-gestatio embryo[5-8], but is unique in that its highest level of expression i the adult animal is restricted to developing male germ cells[5,8,9] We have introduced a construct carrying the mouse *Hox-1.4* gen into the germ line of mice to begin to identify the *cis*-actin elements required for proper expression and to assess the con sequences of increasing *Hox-1.4* gene expression. The construc was designed to produce normal Hox-1.4 protein from transcript that are distinguishable from the products of the endogenous gene The integrated transgene seemed to exhibit the appropriate tissu specificity of expression, but transcript levels were elevated i certain tissues, particularly the embryonic gut. This overexpressio correlated with changes in the normal developmental program o the gut, resulting in an inherited abnormal phenotype known a megacolon.

Hox-1.4 is a member of a cluster of at least six homoeobox containing genes occupying about 100 kilobases (kb) of con tiguous DNA on mouse chromosome 6 (refs 8, 10, 11). Althoug the putative translation stop codon and other structural feature of the 3′ end of the *Hox-1.4* gene have been determined, the 5 end has not been characterized. We therefore generated a con struct (Fig. 1*a*) which contained ~10 kb of sequence upstrean of the homoeodomain and abuts the upstream *Hox-1.* gene[10,12,13].

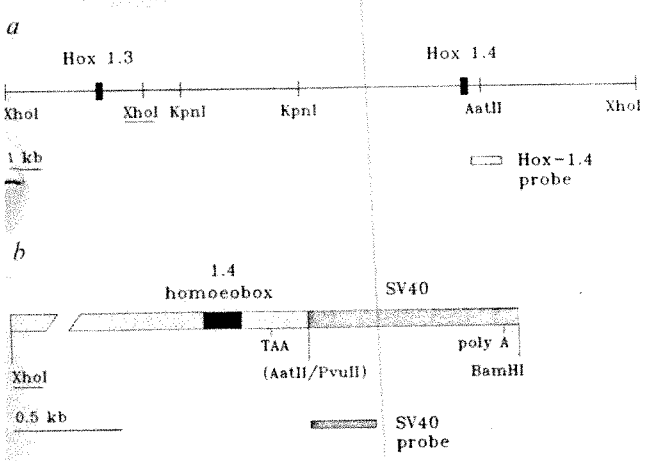


Fig. 1 Map of *Hox-1.4* and construct used to generate transgenic mice. *a*, Map of the *Hox-1.4* gene and flanking regions. Pertinent restriction sites are indicated. *b*, The *Xho*I–*Bam*HI fragment that was microinjected to generate transgenic mice.
Methods. A plasmid spanning *Hox-1.3* and *Hox-1.4* was cut at the unique *Aat*II site to remove the endogenous poly(A) site of *Hox-1.4* (ref. 20). A *Pvu*II–*Bam*HI fragment of SV40 (position 3,506 to 2,533) containing a polyadenylation signal[21] was ligated to the *Aat*II site, downstream of the translation stop codon of *Hox-1.4*(refs 9,20). The 'Hox-1.4 probe' in *a* was used to detect endogenous mRNA[5,20]. To detect transgenic mRNA only, the SV40 probe (Fig. 1*b*) was used. Both fragments were subcloned into pGEM vectors purchased from Promega.

We assumed that this DNA fragment contains the complete coding sequence of *Hox-1.4* and would be capable of producing functional Hox-1.4 protein if expressed. To distinguish the transcripts generated by this construct from those of the endogenous *Hox-1.4* gene, about 400 base pairs (bp) of 3′ untranslated region of *Hox-1.4* was substituted with simian virus 40 (SV40) T-antigen coding region and poly(A) addition site (Fig. 1*b*). Transgenic mRNAs should be ~400 nucleotides (nt) longer than endogenous *Hox-1.4* transcripts and easily distinguishable because of the SV40 sequences.

Initially, we characterized two transgenic lines (1974-3 and 1975-2), each carrying multiple copies of the 10 kb Hox-1.4-SV construct at a single integration site. Inheritance of the transgenes in the 1974-3 lineage is exclusively by males and is therefore genetically linked to the Y chromosome, whereas the pattern of inheritance in the 1975-2 lineage is autosomal.

The tissue specificity and level of expression of the *Hox-1.4* transgene in adult tissues from both lines was examined by northern blot hybridization analysis. SV40-containing mRNAs were readily detected in testes of the 1975-2 lineage, but not in the other adult tissues examined (Fig. 2*a*). Occasionally, a low level of SV40-containing mRNA was detected in lung from these transgenic mice, consistent with the low level of expression of the endogenous *Hox-1.4* gene observed in this tissue (R. Krumlauf, personal communication). Recent preliminary data on the pattern of expression of Hox-1.4-SV constructs with 5 kb (rather than 10 kb) of DNA 5′ to the *Hox-1.4* homoeobox reveal this same pattern of tissue distribution of transgenic mRNAs in the adult animals—highest levels in the testis and barely detectable levels in lung and kidney (another somatic tissue exhibiting low levels of endogenous *Hox-1.4* expression: R. Krumlauf, personal communication; C. M. Viviano and D. J. Wolgemuth, unpublished observations) (data not shown). These observations suggest that the *cis*-acting regulatory elements responsible for the appropriate tissue-specific expression of *Hox*-1.4 are present in these constructs. The levels of Hox-1.4-SV transgenic transcripts however, in the testes of adult males of the 1975-2 lineage (Fig. 2*b*), as well as in testes of two additional lines carrying the 10 kb

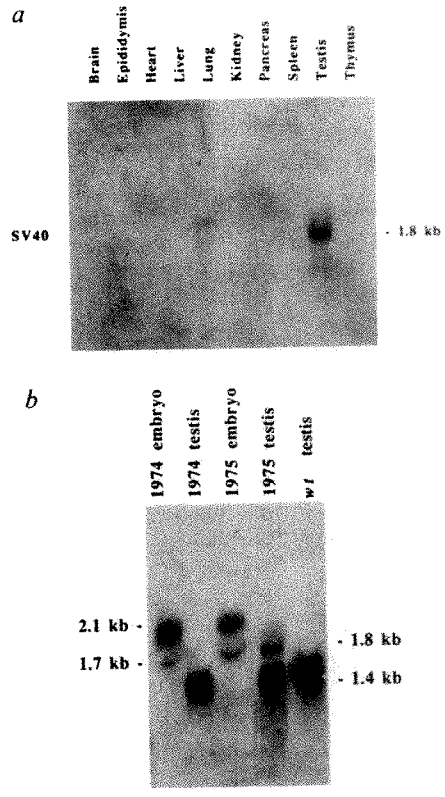


Fig. 2 Northern blot hybridization analysis of expression of the 10 kb Hox-1.4-SV transgene. *a*, Expression of the 10 kb Hox-1.4-SV transgene in adult tissues of the 1975-2 lineage. Total RNA (25 μg) from tissues of an adult (>45-day-old) male mouse from the 1975-2 lineage was examined for the presence of transgenic mRNA using the SV40 probe. Transgenic mRNAs of the predicted ~1.8 kb size were readily detected in testis but not reproducibly in other tissues. *b*, Expression of transgenic and endogenous *Hox-1.4* transcripts in mid-gestation embryos and adult testes of the 1975-2 and 1974-3 lineages. RNA was isolated from transgenic day-12.5 (day 0.5 is day of plug) whole embryos or from testes of adult animals. Total RNA (~25 μg) was loaded per lane. We used the *Hox-1.4* probe, which detects both transgenic and endogenous *Hox-1.4* transcripts (see Fig. 1). The larger transcripts were transgenic, as confirmed by hybridization with the SV40 probe (data not shown). Transcripts of 1.7 and 1.4 kb were produced from the endogenous gene in day-12.5 embryos and adult testes, respectively, as expected[5]. Transgenic mRNAs of ~2.1 and 1.8 were detected in embryo and testis RNA, respectively, from the 1975-2 lineage, whereas the 1974-3 lineage expressed the transgene only in embryos, not in testis.
Methods. Transgenic mice were generated by microinjection of the 10kb Hox-1.4-SV construct into the pronuclei of C57B1/6 × SJL F_2 fertilized eggs by standard prodecures[22]. Weanlings were identified as transgenic by dot hybridization of DNA collected from tails[22]. Embryos were identified by analysis of DNA from placenta and extra-embryonic membranes. Tissues were obtained from progeny of founder animals, unless otherwise stated. RNA was isolated by the LiCl precipitation method[23]. Poly(A)$^+$RNA was isolated as described[24]. Total or poly(A)$^+$ RNA was electrophoresed on 0.8% agarose/2.2 M formaldehyde gels[25], transferred to Genescreen Plus (New England Nuclear), cross-linked under UV light for 7 min, and hybridized at high stringency[25] as prevously described[26]. Isolated inserts were labelled by nick translation with ^{32}P[dXTP][27]. Final washes were at 0.1 × SSC, 68 °C. Filters were exposed to XAR film with intensifying screens.

Hox-1.4-SV transgene and two lines carrying the 5 kb Hox-1.4-SV transgene (data not shown), were lower than those produced by the endogenous gene. This suggests that elements involved in quantitative regulation of *Hox-1.4* mRNA levels may be missing.

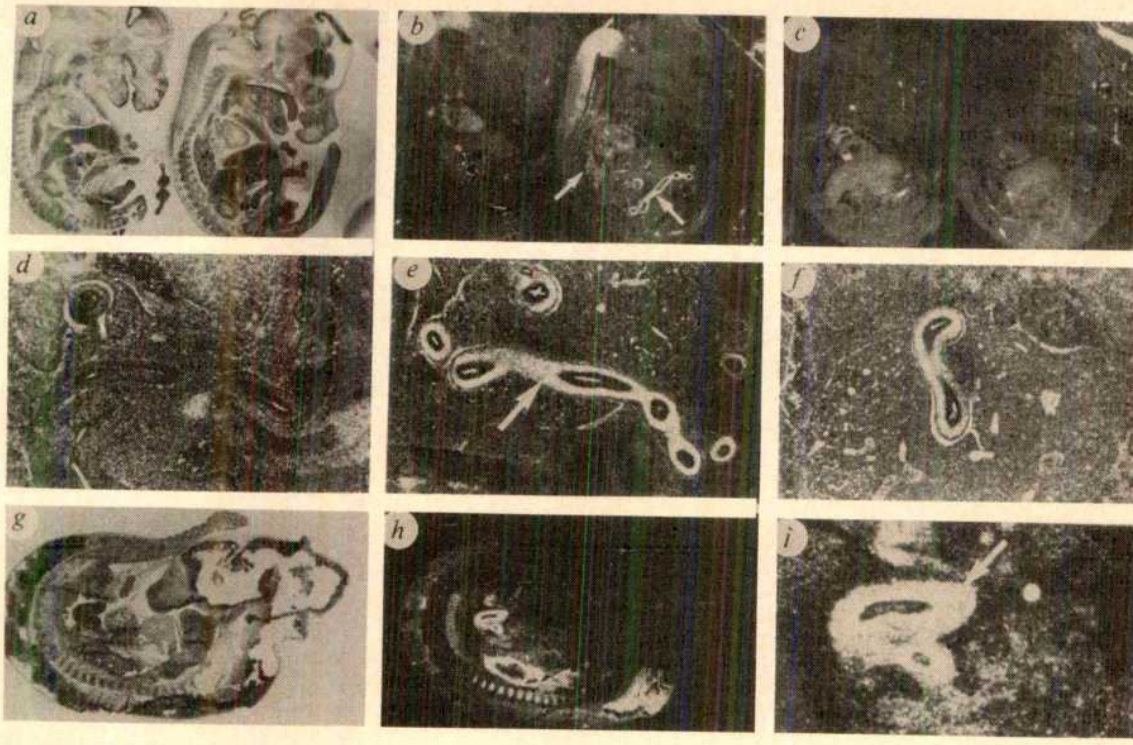


Fig. 3 *In situ* hybridization analyses of endogenous and transgenic *Hox-1.4* transcripts in mid-gestation embryos. *a*, *b*, Parasagittal sections through day 12.5 embryos of the 1974-3 lineage photographed with bright-field (*a*) or dark-field (*b*) optics. The embryo on the left is a non-transgenic litter-mate of the transgenic embryo on the right. The antisense *Hox-1.4* probe used detects both endogenous and transgenic *Hox-1.4* mRNAs. Localization is clearly seen in the more rostral region of the spinal cord and in the caudal myelencephalon of the wild-type and transgenic embryos. The labelling is more intense in the transgenic mouse, indicating higher levels of *Hox-1.4* mRNA. Elevated levels of expression are also evident in the lung and intestine of the transgenic embryo (arrows). The labelling over the heart is non-specific, as heart is also labelled using the sense (control) probe (*c*). The hybridization pattern of antisense *Hox-1.4* (*d*, *e*) or SV40 (*f*) RNA in regions of the developing gut of wild-type (*d*) and transgenic (*e*, *f*) embryos are shown at higher magnification in *d*, *e*, and *f*. Labelling is restricted to the mesenchymal layer of the developing gut (arrows). In the transgenic embryo, the SV40-containing transgenic mRNAs are readily detected and exhibit localization to the mesenchymal layer (*f*). Expression of transgenic *Hox-1.4* mRNA is also elevated in gut of mid-gestation (day 11.5) embryos of the 1975-2 lineage (*g*–*i*). *g*, A bright-field photograph of the embryo in *h*; *i*, an enlargement of portions of the gut. The labelling is again restricted to the mesenchymal layer (arrows in *i*).

Method. Procedures followed previous protocols[6,28], except where noted. Embryos were recovered at day 11.5 and 12.5 of gestation, fixed overnight at 4 °C in 4% paraformaldehyde: 0.1 M phosphate buffer, *p*H7.5, followed by equilibration in 30% sucrose in 0.1 M phosphate buffer, *p*H 7.5. Specimens were embedded in O.C.T. compound (Miles) and sectioned on a cryostat. Sections were collected onto poly-L-lysine coated slides. Hybridization conditions were as described[9,28], except that 10 mM dithiothreitol was included in the first post-hybridization wash. RNA probes were synthesized from the T7 or SP6 promoters in the GEM3 plasmid vector to generate antisense or sense RNA using ^{35}S[UTP]. Specimens in panels (*b*, *d*, *e*) were hybridized with antisense *Hox-1.4* probe panel (*c*) with sense *Hox-1.4* RNAs; panels (*f*, *h*, *i*) with antisense SV40 RNA probe.

There was no detectable expression of the transgene in testes (Fig. 2*b*) or other adult tissues tested (data not shown) of the Y-linked 1974-3 lineage. The lack of expression of the transgene in the Y-linked lineage may be due to the condensation during meiotic prophase of the X and Y chromosomes into the sex vesicle, which is purported to be transcriptionally inactive[14,15].

The northern blot analysis further revealed that in mid-gestation embryos of both the 1974-3 and 1975-2 lineages, the levels of transgenic mRNAs were at least fivefold higher than the levels of the endogenous *Hox-1.4* transcripts (Fig. 2*b*). To determine whether the elevated levels of the transgenic mRNAs in the embryos could be attributed to the presence of higher levels of transcripts in tissues normally expressing *Hox-1.4*, to expression of the transgenic *Hox-1.4* in ectopic sites, or to a combination of both, mid-gestation embryos of the 1974-3 and 1975-2 lineages were examined by *in situ* hybridization analysis.

The endogenous *Hox-1.4* gene is expressed at high levels in the developing nervous system of the mid-gestation mouse embryo, especially within the spinal cord, as shown by both northern blot[5] and *in situ* hybridization analysis[6,7]. Both the non-transgenic and transgenic 12.5-day-old embryos shown in Fig. 3*b* exhibit the expected abundant expression of *Hox-1.4* in portions of the spinal cord and myelencephalon (Fig. 3*a*, *b*). Hybridization signals were also observed in heart. As the signal detected in heart was also observed in experiments using sense *Hox-1.4* probes, however (Fig. 3*c*), it presumably represents non-specific, artefactual hybridization.

In the transgenic embryo, the spatial distribution of *Hox-1.4*-containing mRNAs in the myelencephalon and spinal cord was similar to controls, but the levels seemed elevated (Fig. 3*b*). *Hox-1.4*-containing mRNAs were more readily detected in the lung of the transgenic rather than non-transgenic embryo, suggesting that there are slightly elevated levels of expression in this tissue as well (Fig. 3*b*). We believe that the levels of *Hox-1.4* mRNA are elevated at least twofold in both the central nervous system and lung of the transgenic embryos because the grain density was consistently higher and the time required to obtain a signal was consistently shorter than with litter-mate controls using the *Hox-1.4* probe. In addition, these elevated levels are due to expression of the transgene as both tissues hybridized with the SV40 probe (Fig. 3*h* and data not shown).

Most striking of all was the intense labelling observed in the gut of transgenic embryos; little or no labelling above background was observed in the gut of non-transgenic embryos. This

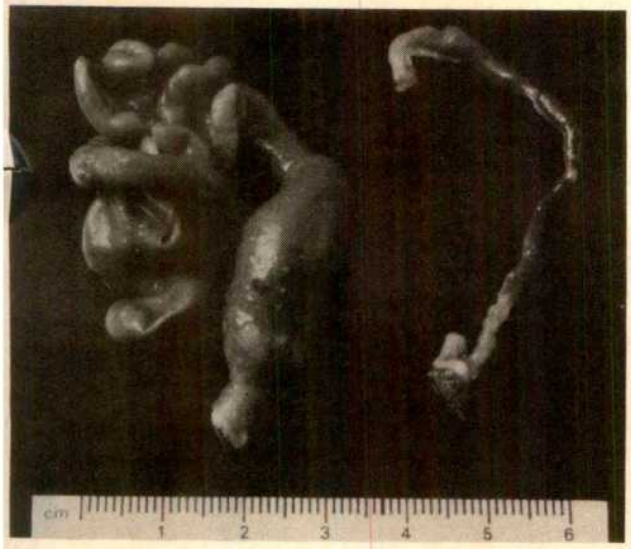


Fig. 4 Example of the megacolon phenotype from the 1975-2 lineage. Left, the dissected colon and a portion of the intestinal region of an adult mouse exhibiting profound megacolon. Right the colon of a normal adult mouse. The distention begins ~0.5 cm from the most distal region of the colon.

labelling was observed in both the 1974-3 (Fig. 3*b–e*) and 1975-2 (Fig. 3*h, i*) lineages. Hybridization with the SV40 probe confirmed that the transcripts observed in the gut were due to expression of the transgene (Fig. 3*f, h, i*). The transcripts were clearly localized to the region of mesenchyme in an evenly distributed, non-punctate pattern. No labelling above background was observed in the inner (mucosa) or outer (serosa) layers. Although it was not evident in the *in situ* hybridization experiments shown in Fig. 3, *Hox-1.4* mRNA has been detected by *in situ* analysis in the mesenchyme of the gut and lung of normal day-12.5 embryos (D. Duboule, S. Gaunt and R. Krumlauf, personal communication). Thus, the elevated expression of the *Hox-1.4* transgene probably represents overexpression of *Hox-1.4* in cells that normally express this gene, rather than ectopic expression.

The highly elevated levels of *Hox-1.4* in the developing gut of both the 1974-3 (Fig. 3*a–f*) and 1975-2 (Fig. 3 *g–i*) lineages are particular interesting because of the unexpected phenotype that develops in these mice. Our initial analysis concentrated on the 1974-3 lineage because the animals were relatively healthy and bred well. The 1975-2 lineage was quite difficult to establish. We determined that the founder male was a germ-line mosaic, transmitting the transgene to ~20% of the embryos. Only two of more than 100 weanlings generated from this founder animal carried the transgene, however. These observations suggested that transgenic pups were dying between birth and young adulthood; we have subsequently determined that they succumbed to a condition resembling congenital megacolon[16]. Mice with megacolon have difficulty extruding faeces through the most distal portion of their colon, resulting in a grossly distended bowel, compacted faeces and, in many cases, death. An example of this phenotype is illustrated in Fig. 4. This condition varied in severity and therefore most animals lived long enough to reproduce either on their own (occasional males) or via superovulation and embryo transfer. The original 1975-2 founder male eventually succumbed to megacolon when 11 months old.

Every transgenic animal examined in the 1975-2 lineage exhibited the megacolon phenotype. We have also observed the megacolon phenotype in two animals from the 1974-3 line and in four lines of transgenic mice carrying the 5 kb Hox-1.4-SV constructs. Preliminary observations on expression of the transgenes in one of these 5 kb lines have revealed elevated levels of *Hox-1.4* expression in the mid-gestation gut (data not shown). The megacolon phenotype varied in severity among the various lines, with the 1974-3 lineage rarely showing symptoms to one of the 5 kb Hox-1.4-SV lines in which all progeny carrying the transgene exhibited megacolon soon after birth and died before they were 10 days old. Multiple lines of transgenic animals exhibited a similar phenotype, indicating that the alteration in gut development was due to expression of the transgene, and not to a dominant insertional mutation.

There are several mouse mutant strains that exhibit megacolon as either homozygotes (for example, piebald lethal and lethal spotting[17]) or heterozygotes (dominant megacolon[18]). A congenital condition known as Hirschsprung's disease results in megacolon in man[19]. In each of these conditions the affected individuals exhibit a deficiency of myenteric ganglion cells in the colon[16]. These are neural crest derivatives that migrate into the developing gut to innervate the bowel[16]. The defect which results in megacolon may therefore be intrinsic to the neural crest cells; alternatively, the defect may lie in cells in the various layers of the developing gut that provide cues to the migrating neural crest cells. It is thus noteworthy that the *Hox-1.4* transgene is overexpressed in the mesenchymal layer of the day-12.5 embryonic gut of the transgenic mice.

It seems that the increased level of *Hox*-1.4 mRNA in the transgenic embryos is due to varying levels of elevated expression of the transgene at the appropriate sites, rather than to ectopic expression. Although there could be effects of the slightly elevated levels of *Hox-1.4* mRNAs in the lung and CNS, we have not observed any obvious developmental defects. In contrast, the development of megacolon in adult mice correlates with high levels of *Hox-1.4* transcripts in the embryonic gut and constitutes the first example of a mutant phenotype associated with the expression of a mammalian homoeobox-containing gene.

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In vivo binding pattern of a *trans*-regulator of homoeotic genes in *Drosophila melanogaster*

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The specification and maintenance of the metameric pattern in *Drosophila melanogaster* is regulated by complicated gene interactions. The differential expression of the homoeotic genes of the *Antennapedia* complex (ANT-C) and *bithorax* complex (BX-C), which determine segmental identities, is partly controlled by cross-regulatory interactions of loci within the two clusters and partly by *trans*-acting factors located outside the two complexes. One of the *trans*-regulatory genes, *Polycomb* (*Pc*), acts as a repressor of the ANT-C and BX-C[1–4]. Mutations of *Polycomb* result in a complete derepression of the homoeotic genes, leading to abdominal transformations of all body segments. *Polycomb* is part of a large class of *trans*-regulatory genes (*Pc*-group), estimated to comprise up to 40 loci[5]. We have raised antibodies against the Polycomb protein, and, using an improved immunostaining tech nique, showed that the Polycomb protein binds to 60 discrete sit along the polytene chromosomes of salivary glands. These sit comprise the ANT-C and the BX-C as well as several locatio of *Pc*-group genes. This is the first clear evidence for a dire interaction of *Polycomb* with homoeotic loci and other *Pc*-grou genes.

The *Polycomb* locus localizes to polytene chromosome ban 78 D 7,8. The DNA encompassing the gene has been cloned b a microdissection technique (R.P., J. Lauer and D. S. Hognes manuscript in preparation). About 4 kilobase (kb) of DN enclose the entire *Pc* locus, as shown by P-element-mediate germline transformation. *Polycomb* encodes three different tra scripts. Poly(A)$^+$ transcripts of 2.5 kb and 2.0 kb are very abu dant early in development (0–6-h-old embryos). The 2.5 kb RN is also transcribed during the remaining embryonic, larval an pupal stages, although at lower levels. A 1.0 kb transcript i especially abundant in late third instar larvae: in larval salivar glands it is the only *Pc* transcript found. Several complet cDNAs for the 2.5 kb transcript were isolated . Their sequenc reveals an open reading frame with a coding capacity for a 39 amino-acid Polycomb protein of relative molecular mass (M_r 44,000 (44K) (R.P. and D. S. Hogness, manuscript in prepar ation).

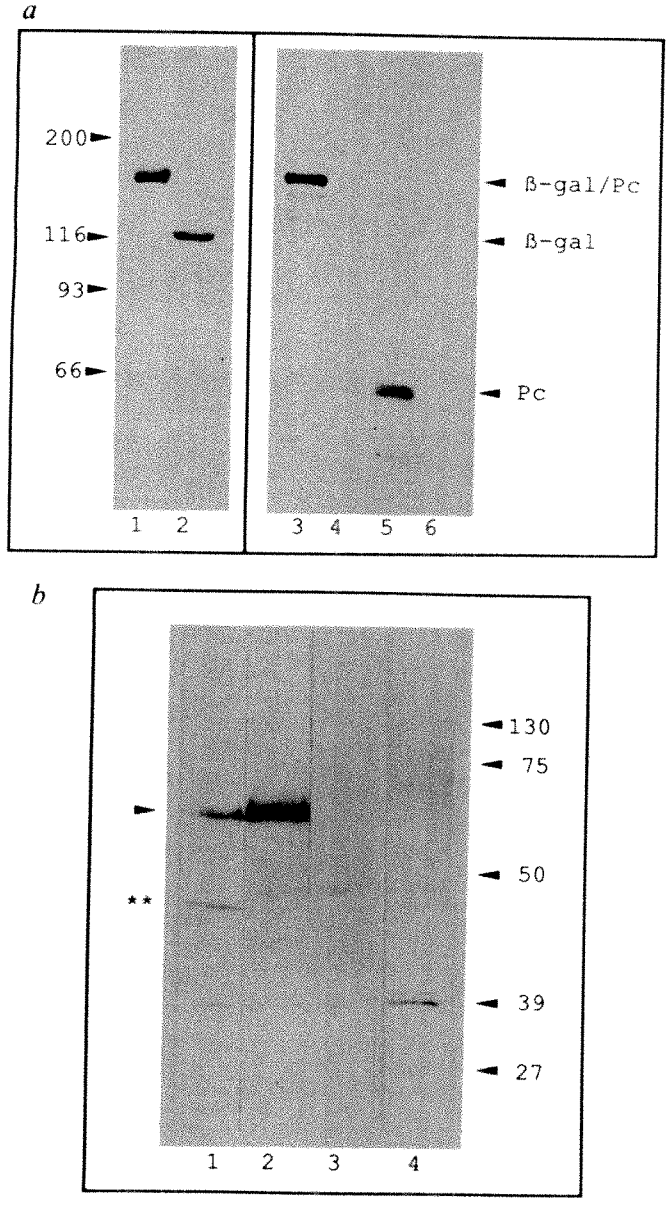


Fig. 1 Antibodies against the Polycomb protein. Polyclonal antibodies were raised against a lacZ–Pc fusion protein containing the C-terminal 199 amino acids of a Polycomb protein encoded by the 2.5 kb transcript. *a*, Lanes 1 and 2: partially purified βgal-Pc and βgal, respectively, were separated on a SDS-PAGE gel and stained with Coomassie Blue. Lanes 3 and 4: immunoblotted proteins equivalent to those in lane 1 and 2, respectively. Lane 5: a T5 expression vector (pUHE 21-3)[21] was used to express the authentic Pc protein in *Escherichia coli*. Crude extracts were separated on a SDS-PAGE gel and immunoblotted. Lane 6: immunoblotted crude *E. coli* extract harbouring the T5 vector only. Size markers are indicated on the left in thousands. *b*, *Drosophila* protein extracts were separated on SDS-PAGE gels and transferred to nitrocellulose. Western blots were incubated with purified polyclonal anti-Pc antibodies (lanes 1–3). Lane 1: extract from 150 third instar larval salivary glands. The lower band (**) shows the smaller, salivary gland-specific Pc protein. The 44K Pc protein is also visible (arrow), resulting from contaminating fat body cells (see also Fig. 2*a*). Lane 2: extract from 300 wild-type embryos staged between 18–22 hours. Only the predominant 44K Pc protein is visible. Lane 3: extract from 300 homozygous Pc^K-mutants staged between 18–22 hours, showing the absence of the specific 44K Pc band. The Pc^K-mutant was found to have a 2.0 kbp deletion in the Pc gene (R.P., J. Lauer & D. S. Hogness, manuscript in preparation). Lane 4: same extract as in lane 2 except that the western blot was incubated with the second antibody only. Size markers are indicated on the right, in thousands. The 44K Pc protein and presumably also the smaller Pc protein migrate aberrantly on a SDS-PAGE gel compared to the calculated M_r deduced from the cDNA sequence.

Methods. A 920-base pair (bp) cDNA fragment was cloned into the *Bam*HI site of pUR278 (ref. 22). BMH71-18 host cells were grown and induced as described[23]. The preparative purification of the 150K fusion protein was accomplished by urea extraction of the insoluble aggregate as described[23]. Out of one litre of culture we obtained 20–40 mg fusion protein, which was 90% pure. Rabbits were immunized with 100 μg fusion protein in PBS/complete Freund's adjuvant and boosted with 40 μg antigen in PBS/incomplete Freund's adjuvant. For the purification of anti-Pc antibodies crude antiserum was loaded on a β-galactosidase-Affigel 15 column (equilibrated in 0.1 M Tris-Cl *p*H 8). The flow-through was passed over a β-galactosidase-Pc-Affigel 15 column (equilibrated as above). This column was washed with 0.1 M Tris-Cl *p*H 8, 0.5 M NaCl. Antibodies were eluted with 0.1 M glycine-Cl *p*H 2.5. The specificity of the anti-Pc antibodies was checked by western blots of Pc proteins produced in *E. coli* (see Fig. 1*a*, lanes 3–6) and to *Drosophila* protein extracts (see Fig. 1*b*). *Drosophila* extracts were prepared as described[24].

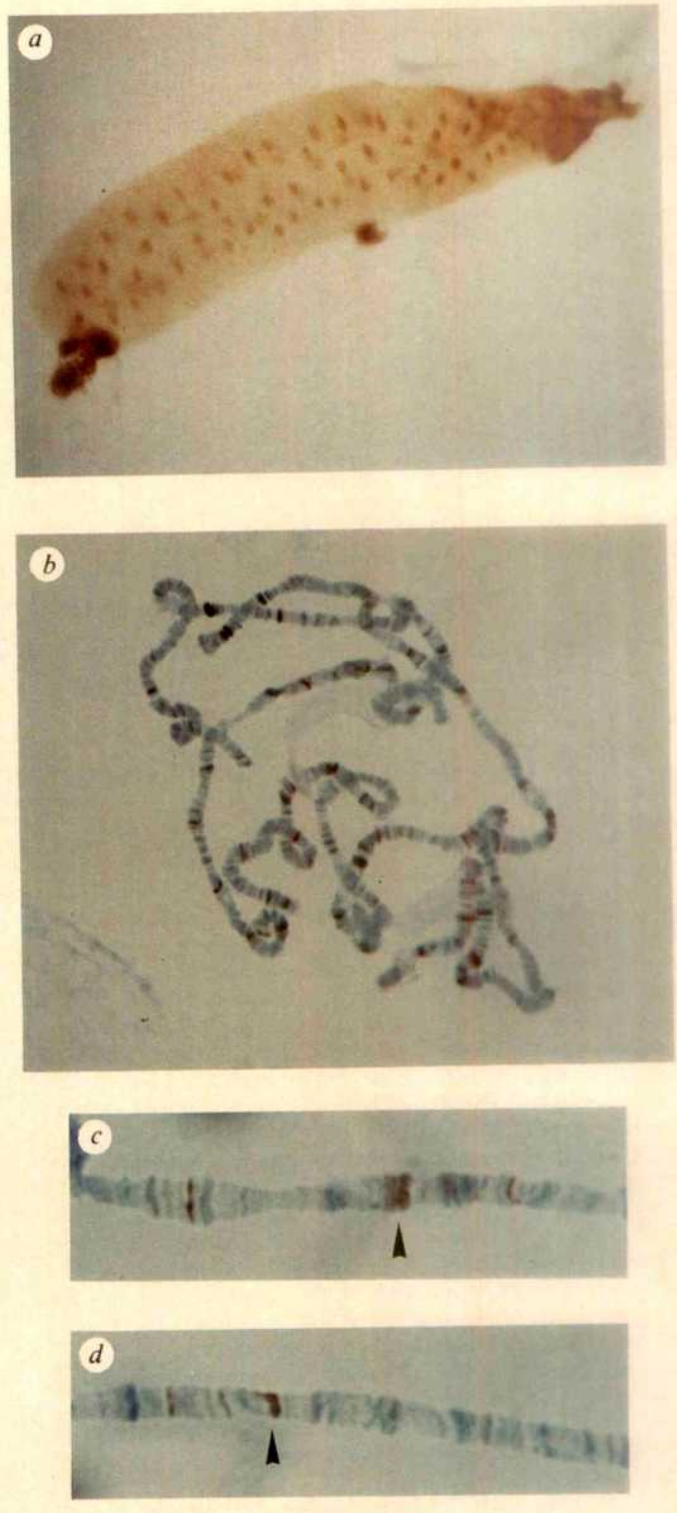


Fig. 2 Immunohistochemical localization of the Pc protein in salivary glands and on polytene chromosomes of third instar larvae. *a*, Single salivary gland showing intense staining of the large polytene nuclei. The tip of the gland shows strong staining of fat body cells. *b*, Immunostaining of squashed salivary gland chromosomes. The Pc protein is found associated with about 60 chromomeres (see Table 1) distributed over all chromosomes. No Pc protein is found in the centromere and in the nucleolus. *c*, Section of the 3rd chromosome showing the localization of the ANT-C (84 A 4,5–B 1,2; arrow). The most intense labelling is found in the distal portion of the complex (84 B 1,2). *d*, Section of the 3rd chromosome showing the association of Pc protein with the BX-C (89 E, arrow). The proximal-distal orientation of the chromosomes in *c* and *d* is from left to right.

Methods. Immunostaining of whole salivary glands. Salivary glands were dissected in PBS, 0.1% Triton X-100, fixed for 5 min in buffer A (100 mM HEPES *p*H 7.0, 2 mM $MgSO_4$, 1 mM EGTA) containing 1% formaldehyde. Glands were then washed several times with buffer A and then with BBT *p*H 7.0 (10 mM Tricine, 55 mM NaCl, 40 mM KCl, 7 mM $MgCl_2$, 5 mM $CaCl_2$, 20 mM glucose, 50 mM sucrose, 0.1% BSA, 0.2% Tween20). Glands were incubated for 1 h in BBT containing affinity-purified anti-Pc antibody, diluted to the desired concentration. Primary antibody incubation was followed by rinsing the glands in BBT. Then anti-rabbit IgG biotinylated antibody (preabsorbed to embryos; 1:1,000 dilution; Vector) in BBT, 1% normal serum was added for 40 min. Glands were then rinsed as above, followed by several short rinses in PBT (PBS, 0.1% Tween20). PBT was then replaced by PBT containing 1% reagent A (Avidin DH), 1% reagent B (biotinylated HRP) (both Vectastain ABC kit, Vector), which had been preincubated for 30 min. After a 40 min incubation the glands were washed again in PBT. Glands were stained with PBT, 0.005% DAB, 0.025% H_2O_2. The staining reaction was stopped by several washes in PBS. Salivary glands were mounted in 50% glycerol, PBS. Immunostaining of polytene chromosomes. Dissected salivary glands were transferred into a droplet of 3.7% formaldehyde, 1% Triton X-100, PBS *p*H 7.5 for 30 s. The glands were then moved in a 40 μl drop of 3.7% formaldehyde, 50% acetic acid on a coverslip for 2–3 min. Glands were squashed, the slides frozen in liquid nitrogen and the coverslip flipped off with a razorblade. The slides were rinsed in PBS, followed by BBT. The immunostaining procedure was essentially the same as for the glands. For all the incubation steps, the slides were kept in a humid atmosphere with a coverslip placed over the chromosomes. After immunostaining, chromosomes were stained for about 15 s in a 1:100 diluted Giemsa solution. After brief washing in distilled water, the chromosomes were embedded in 99.5% glycerol and immediately examined under the microscope. Photographs were taken on Kodak Ektachrome film using a Zeiss Axioplan microscope.

We have raised antibodies to the 199 C-terminal amino acids of the Polycomb protein. Polyclonal antiserum was purified and used to detect the protein in extracts of wild-type embryo (see Fig. 1*b* lane 2). The specificity of the antiserum is shown by the absence of the Pc protein band in western blots of extracts from mutant embryos (see Fig. 1*b*, lane 3). In addition, we have visualized the embryonic distribution of the Pc protein by immunostaining of whole mount embryos (B.Z. and R.P., manuscript in preparation). No immunostaining was seen in homozygous Pc^--mutant embryos (data not shown).

The expression of a specific *Pc* transcript in salivary glands of third instar larvae encouraged us to analyse this tissue for the presence of the Pc protein. Salivary glands contain large polytene chromosomes, which greatly facilitate the analysis of gene interactions. Figure 2*a* shows the specific labelling of the large nuclei of a salivary gland by the anti-Pc antibodies. The salivary gland-specific 1.0 kb transcript does not have sufficient coding capacity for a 390 amino-acid protein. It encodes a smaller Pc protein, which is also specifically recognized by the antiserum (Fig. 1*b*, lane 1). Spreading and immunostaining of the polytene chromosomes revealed that this Pc protein is associated with approximately 60 sites (Fig. 2*b* and Table 1). Cytological identification of the binding sites showed that Pc is associated with loci shown to interact with *Pc* by genetic methods (see Table 2). Pc protein is abundantly found at the ANT-C (84 A 4,5–B 1,2) and at the BX-C (89 E) (Fig. 2*c*, *d*). The strong

Table 1 Pc Binding sites

X Chromosome	2nd Chromosome	3rd Chromosome	4th Chromosome
1 B	21 AB	61 C 1,2	102 D 1
2 D 1-4	22 A	62 E	102 C 1
4 C 1,2	22 C	63 A	
5 A	24 A	65 D	
5 D 1,2	25 EF	67 D	
7 B	26 F	67 F	
8 A	29 DE	68 E	
9 B 5-8	32 EF	69 C 1	
14 A	33 F	69 D 1,2	
14 F	35 C	70 A	
	36 B	82 E 1	
	38 F	84 A4,5-B 1,2	
	41 CD	84 D 1,2	
	43 C 1	84 F 1,2	
	44 A	85 E 1	
	48 A	86 C 1,2	
	48 C	88 A 1,2	
	49 E 4-7	89 B 1,2	
	51 A	89 C	
	56 BC	89 E	
	56 F6-9	90 E	
	59 EF	93 E 1-4	
	60 E1	96 A 1,2	
		98 A	
		99 EF	

Location of Pc binding sites on salivary gland polytene chromosomes of *Oregon-R* wild-type larvae. The cytological positions of the binding sites were determined by comparison of the polytene bands to the map of Bridges[14].

Table 2 Pc Binding to cytologically mapped regulatory genes

	Cytology*	Binding sites of Pc
Homoeotic genes		
ANT-C	84 A 4,5-B 1,2	84 A 4,5-B 1,2
BX-C	89 E	89 E
Pc-group genes		
Additional sex combs (*Asx*)	51 AB	51 A
Extra denticles (*exd*)	13 F 1-14 B 1	14 A
Polycombeotic	67 F	67 F
Polyhomeotic (*ph*)	2 D 2-3	2 D 1-4
Posterior sex combs (*Psc*)	49 EF	49 E 4-7
Sex combs on midleg (*Scm*)	85 EF	85 E 1
Super sex combs (*sxc*)	41 C	41 C
Pc-interacting genes		
Enhancer of (*Pc*)	48 A	48 A
Suppressor of (*Pc*)[1]	21 AB	21 AB
Suppressor of (*Pc*)[2,3]	35 CD-38 B	35C+36 B
Suppressor of (*Pc*) (Brista^{MP})	60 D 14-E 2	60 E 1

Location of selected Pc binding sites that overlap with cytologically mapped genes with which Pc is interacting. Cytological positions (*) were taken from the following references: ANT-C (ref. 15); BX-C (ref. 1); *Asx*, *Psc* and *Scm* (ref. 5); *exd* (ref. 16); *polycombeotic* (A. Shearn, personal communication); *ph* (ref. 17); *sxc* (ref. 18); *E(Pc)* (ref. 8); *Su(Pc)*^{1,2,3} (ref. 9); *Su(Pc)(Brista*^{MP}) (refs 9, 19). 48A is also the location of the *engrailed* gene (ref. 20), as Pc was shown to interact with *engrailed* also (G. Morata, personal communication).

Pc antibody staining at the positions of these two complexes may reflect the regulatory activity of *Polycomb* on the large number of homoeotic genes located in these two clusters. In addition, Pc antibody staining is associated with several cytologically identified locations of *Pc*-group genes. Major exceptions were the *esc*[6] and the *Pcl*[7] loci, where there was no binding of the Pc protein. A very prominently labelled site was found at 49E 4-7, the presumptive location of *Psc*[5]. Pc labelling was also found associated with sites to which a third class of genes has been mapped[8,9]. These genes were found to interact with *Polycomb*, by either enhancing or suppressing the posterior transformations observed in *Pc*$^-$ mutants. They do not cause a *Pc*-like phenotype on their own, however, and their physiological function in development is unknown.

Table 2 shows the sites of Pc antibody labelling at locations of mapped genes, previously shown by genetic analysis to interact with *Pc*. The resolution of the cytological mapping is obviously limited by the size of the chromosomal aberrations used to define the location of a gene. Because the binding sites overlap with several members of a certain class, however, it is highly probable that Pc does indeed bind and regulate the genes indicated in Table 2.

Salivary glands are ectodermally-derived accessory organs of digestion and glue secretion, developing from an invagination of the labium[10]. No expression of homoeotic genes in this type of tissue has been reported so far. The presence of the Pc protein, acting as a repressor, would be consistent with this result. Although the binding pattern on polytene chromosomes of salivary glands can only represent the status of this particular tissue, genetic evidence has shown that Pc interacts with the same genes in other ectodermally-derived tissues[1-4,11-13]. This suggests that the pattern seen in salivary glands is unlikely to be completely different from that expected for other types of tissue. The terminal differentiation of larval salivary glands, which histolyse at metamorphosis, might indicate a default setting of *Polycomb*, keeping all homoeotic genes in a repressed state. At earlier developmental stages *Polycomb* might, with the help of different sets of *Pc*-like genes, repress homoeotic genes in a segment- and tissue-specific way.

This work demonstrates the first complete *in vivo* chromosome-binding pattern of an important regulatory factor involved in metameric development. We have shown that the Pc protein binds to genes shown genetically to interact with *Pc*. In addition we have identified the cytological location of other target genes of *Pc*. It will be interesting to define the integration of *Polycomb* in this complex regulatory network and to determine whether it acts as a negative regulator of the ANT-C and BX-C, but as an activator of other target genes. In addition it will be necessary to identify how *Pc* exerts its function at the molecular level: whether it regulates genes by acting as a DNA sequence-specific binding factor, or by recognizing other proteins (for example other Pc-group proteins) and interacting as a regulatory complex. To unravel the molecular mechanisms generating pattern formation in *Drosophila* it is necessary to identify as many of the components participating as possible. The use of cytology to study the complicated hierarchical gene interactions involved greatly facilitates this task. Moreover, the technique used here should prove valuable in the identification of target genes for other regulatory factors in *Drosophila* and, potentially, other regulatory elements from different biological systems that can be expressed in *Drosophila*.

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Granulocyte- and granulocyte–macrophage-colony stimulating factors induce human endothelial cells to migrate and proliferate

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Granulocyte-colony stimulating factor (G-CSF) and granulocyte–macrophage-colony stimulating factor (GM-CSF) belong to a family of glycoprotidic growth factors required for the survival, growth and differentiation of haematopoietic precursors and which affect the function of circulating mature cells. They are produced by resting or stimulated stromal cells of the haematopoietic microenvironment (fibroblasts and endothelium) and by immunocompetent cells (T cells and monocytes/macrophages)[1,2]. The action of these CSF molecules was thought to be restricted to cells of haematopoietic origin. Here, we report that G-CSF and GM-CSF influence the migration and proliferation of human endothelial cells suggesting that these molecules may act as regulatory signals outside the haematopoietic system.

When different preparations of human recombinant G-CSF and GM-CSF were tested on human endothelial cells (HEC), a marked increase of migration across polycarbonate filters and incorporation in DNA of [^{3}H-methyl]thymidine ([^{3}H]thymidine) was observed (Fig. 1). Coating with gelatin of polycarbonate filters and plastic wells was necessary to observe HEC migration and [^{3}H]thymidine uptake in response to G-CSF and GM-CSF. Both factors acted in a dose-dependent way. The maximal response elicited by G-CSF and GM-CSF was somewhat lower in all experiments than that elicited by the optimal concentration of fibrinogen used as a chemoattractant in these studies[6] or by human recombinant basic fibroblast growth factor[7] used as growth factor (Table 1). HEC grown for 48 h in 1% fetal calf serum (FCS) with G-CSF or GM-CSF entered the S-phase within 8 h, measured by the incorporation of [^{3}H]thymidine and maximal incorporation occurred between 12 and 16 h, with lower values thereafter (data not shown). Increased DNA synthesis is coupled to a true HEC growth, with a two- to threefold increase in cell numbers after six days of culture with 140 and 250 pM G-CSF and GM-CSF, respectively (three experiments).

Cell proliferation is associated with the expression of a set of genes including c-*myc* and c-*fos*; c-*fos* is activated in response to chemoattractants[8], including G-CSF and GM-CSF[9]. Using northern analysis we detected a marked induction of c-*fos* mRNA in HEC (peak at 2 h) in the presence of cycloheximide (a superinducer of c-*fos* mRNA[10]).

In an attempt to identify the G-CSF and GM-CSF receptors on HEC that are responsible for the unexpected responses evoked by these cytokines in these non-haematopoietic cells, we studied the binding of ^{125}I-labelled molecules to HEC. Figure 2 shows representative experiments of the three performed for each cytokine. High-affinity binding sites were identified on HEC. The receptor number on the cell surface was estimated to be 356 ± 123 and 423 ± 187 with a dissociation constant (K_d) of 21 ± 8 pM and 54 ± 31 pM for G-CSF and GM-CSF, respectively (mean ± s.d. of three experiments). These values are of the same order of magnitude as those estimated for myelocytic or monocytic cells[11-13].

The unexpected effects of G-CSF and GM-CSF on the migration and proliferation of HEC could be due to contaminants present in the recombinant CSF preparations. The first indication that G-CSF and GM-CSF were indeed responsible for these activities was provided by the cytokine specificity of these effects. Monocytic-CSF (Fig. 1*a*) and interleukin 5 (IL-5) (Table 1), an

Table 1 The G-CSF and GM-CSF molecules are active in inducing HEC migration and proliferation

Agent	HEC migration (number of cells)	[^{3}H]thymidine incorporation (c.p.m. × 10^3)
Medium	44 ± 8	1.95 ± 0.86
IL-5 (1:10^3 dilution)	49 ± 6	ND
IL-1 (50 ng ml^{-1})	55 ± 8	ND
G-CSF (200 pM)	72 ± 1*	17.38 ± 3.91*
Boiled G-CSF	38 ± 9	3.21 ± 1.36
G-CSF + polymixin	79 ± 12*	18.31 ± 3.71*
G-CSF + anti-G-CSF	44 ± 5	4.12 ± 2.10
G-CSF + irrelevant IgG	82 ± 14*	16.12 ± 3.12*
Fibrinogen (1 mg ml^{-1})	132 ± 12*	ND
Fibrinogen + anti-G-CSF	129 ± 7*	ND
GM-CSF (250 pM)	85 ± 7*	15.27 ± 3.81*
Boiled GM-CSF	37 ± 6	2.34 ± 1.23
GM-CSF + polymixin	79 ± 9*	15.34 ± 2.81*
GM-CSF + anti-GM-CSF	45 ± 6	3.51 ± 0.88
GM-CSF + irrelevant IgG	86 ± 16*	14.67 ± 1.45*
Fibrinogen (1 mg ml^{-1})	103 ± 12*	ND
Fibrinogen + anti-GM-CSF	104 ± 11	ND
Basic FGF (10 ng ml^{-1})	ND	39.12 ± 4.56*

Recombinant molecules were heat-inactivated by boiling for 10 min. Polymixin B (Sigma) was used at a concentration of 10 μg ml^{-1}. The mouse anti-human-G-CSF monoclonal antibody (mAb) 75A was from K. Welte and C. Bordingnon at a concentration of 8.2 mg ml^{-1} and a neutralizing activity of 33 U μg^{-1}. G-CSF (5 ng ml^{-1}) was preincubated with mAb (38 μg ml^{-1}) at room temperature for 40 min and then tested in a migration and [^{3}H]thymidine incorporation assay. Two different anti-GM-CSF antibody preparations were used in these experiments. The anti-human GM-CSF mAb used in migration experiments was from Dr Kurrle. GM-CSF (5 ng ml^{-1}) was preincubated with the anti-GM-CSF mAb (1.25 μg ml^{-1}) for 40 min at room temperature and then tested in the migration assay. A polyclonal rabbit anti-GM-CSF antibody (1:1,000 dilution) was used in proliferation experiments. The OKT11 mAb (Ortho) or mouse normal IgG were used as irrelevant IgG in these experiments. Human recombinant IL-1 was from Sclavo, Siena, Italy. Fibrinogen (from Dr E. Dejana) and human recombinant basic fibroblast growth factor (FGF) (Amersham) were used as reference agents active on HEC migration[6] and proliferation[7]. For basic FGF-induced [^{3}H]thymidine incorporation, the experiment was performed as described in the Fig. 1 legend, except that an incubation time of 48 h with the growth factor (optimal for FGF) was used. ND, not determined.
* $P < 0.05$ versus medium control.

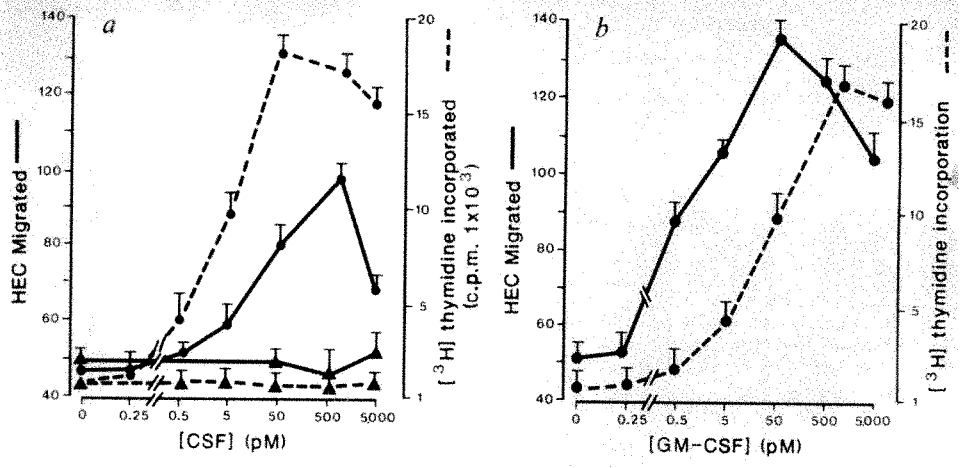


Fig. 1 Induction of HEC migration and proliferation by G-CSF (*a*, ●), monocytic-CSF (M-CSF, *a*, ▲) and GM-CSF (*b*, ●).

Methods. From the human umbilical vein, HEC (III–VIII passage) were cultured as described[3]. The cells (2×10^4 per well, 24 well plates precoated with 0.05% gelatin for 24 h) were grown for 24–36 h in M199 with 20% FCS and then starved for 48 h in 1% FCS with 0.5% bovine serum albumin (BSA, fraction V, Sigma). Medium was then removed and replaced with control medium containing 2% FCS with or without recombinant G-CSF (Dr T. Okabe, 7.8×10^7 U ml^{-1}; or Amgen, 1.5×10^8 U mg^{-1}), GM-CSF (Genetics Institute; or Behringwerke; or Immunex, 10^7 U mg^{-1}) and M-CSF (Cetus, 4×10^7 U mg^{-1}). The cells were incubated for 11 h at 37 °C, [^{3}H-methyl]thymidine (0.5 μCi; 30 Ci mmol^{-1}, Amersham) was added and incubation continued for 1 hour. Medium was removed, cells were washed twice and detached by trypsinization. DNA was precipitated with 10% cold trichloroacetic acid and collected on 0.2 μm Gelman filters[4]. Radioactivity was measured by liquid scintillation counting. Results presented are the mean ± s.d. of three experiments in triplicate. Chemotaxis experiments were performed with a 48-well modified Boyden chamber technique[5,6]. Polycarbonate filters (5 μm pore size, polyvinylpyrrolidone-free) were soaked in 0.5 M acetic acid, washed with PBS, incubated for 24 h in 0.01% gelatin (Sigma) and air-dried. G-CSF, GM-CSF and M-CSF (25 μl) in M199 containing 0.2% BSA were seeded in the lower compartments and 50 μl of HEC (2×10^6 ml^{-1}) were added in the upper ones. After 6 h incubation at 37 °C, the upper surface of the filter was scraped with a rubber policeman. The filters were fixed and stained, and five oil immersion fields (lower surface) were counted after coding the samples. Results are presented as mean ± s.d. migrated cells (statistical significance assessed) by Dunnet's test. Results presented are from individual experiments out of nine (GM-CSF) or three (G-CSF, M-CSF).

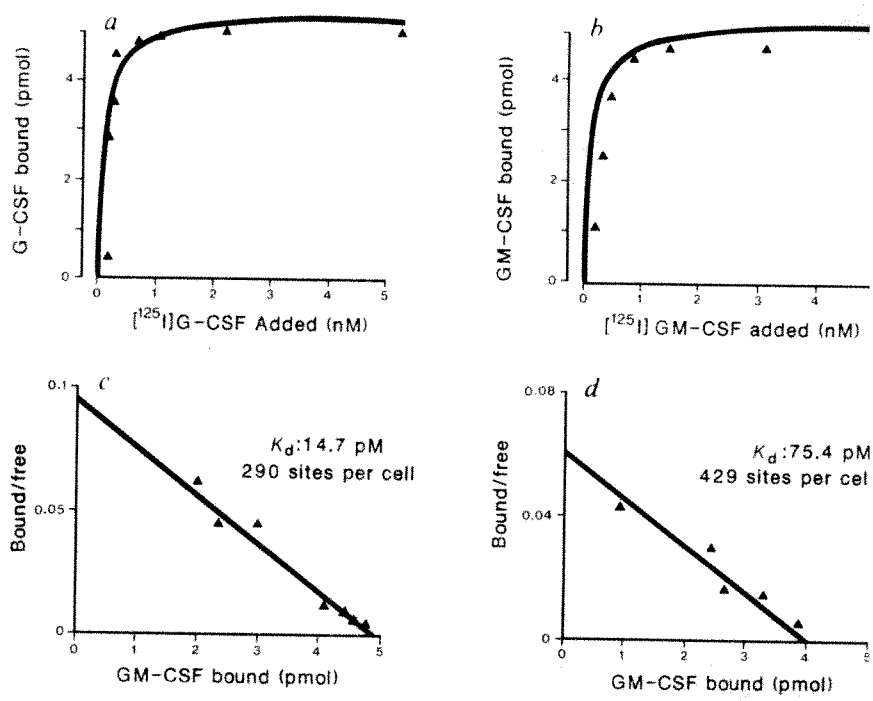


Fig. 2 G-CSF and GM-CSF receptors on HEC. *a*, *b*, Saturation curve at equilibrium: *c*, *d*, Scatchard representation of the specific binding.

Methods. Binding experiments have been performed by using [^{125}I] G-CSF and [^{125}I] GM-CSF. G-CSF and GM-CSF (30 μg per 20 μl borate buffer 0.1 M, *p*H 8) were iodinated using Bolton-Hunter reagent (Amersham; 0.5 mCi for each cytokine). Labelled cytokines were passed througyh Sephadex G-25. The specific activity of the radiolabelled G-SCF (6 ng per 10^5 c.p.m.) and GM-CSF (7.4 ng per 10^5 c.p.m.) was assessed by measuring protein concentration. Iodinated cytokines are active on HEC in terms of migration and proliferation. HEC were detached from the plastic flasks by incubation for 10 min at 37 °C in PBS containing 1 mM [ethylenebis(oxyethylenenitrilo)] tetraacetic acid and then washed twice in medium 199 containing 25 mM (4-(2-hydroxyethyl)-1-piperazinoethanesulphonic acid, *p*H 7.4 and 0.2% BSA (binding buffer). Cells (4×10^6; 95–97% viable) in 0.4 ml of binding buffer containing different concentrations of labelled G-CSF and GM-CSF, with or without 100-fold excess of unlabelled cytokines, were incubated for 2 h at 23 °C. In preliminary experiments, binding at 23 °C required 2 h to reach the equilibrium for both cytokines. The reaction was stopped by centrifugation of the cells, which were overlayered onto 0.8 ml of cold binding buffer containing 75% FCS[11] and the radioactivity in the pellet was determined. Specific binding was determined as the amount of binding inhibited by 100-fold excess of the unlabelled G-CSF or GM-CSF. Binding data were analysed by nonlinear least squares curve fitting with ENZFITTER program (IBM). Data shown are representative of three different experiments done in triplicate.

eosinophil CSF[14], had no effect on the migration and proliferation of HEC. Similarly, IL-1, a molecule active on haematopoietic progenitors[15] and with profound effects on HEC function[16], did not induce HEC migration (Table 1). Thus, the capacity to elicit a migratory and proliferative response in HEC is not shared by all recombinant molecules active on haematopoietic precursors, even when these cytokines, like IL-1, profoundly affect other HEC functions. Polymixin B which inhibits bacterial lipopolysaccharides, did not affect the activity of G-CSF and GM-CSF (Table 1). Boiling abolished the activity of G-CSF and GM-CSF (Table 1). Anti-G-CSF and anti-GM-CSF antibodies abolished the induction of HEC functions (Table 1). Irrelevant control antibodies were inactive and the inhibitory effect of specific antibodies did not seem to be due to non-specific effects, as there was no effect on the activity of fibrinogen. We also tested the possibility that a non-HEC cell

Table 2 Checkerboard analysis of induction of HEC migration by GM-CSF

Experiment 1

	Above			
Below	Medium	0.5 pM GM-CSF	5 pM GM-CSF	50 pM GM-CSF
Medium	74 ± 3	75 ± 2	78 ± 8	82 ± 4
GM-CSF (pM)				
0.5	87 ± 6	77 ± 16	88 ± 1	93 ± 4
5	106 ± 3*	96 ± 6*	95 ± 1*	100 ± 6*
50	110 ± 2*	97 ± 1	115 ± 12*	101 ± 11*

Experiment 2

	Above			
Below	Medium	5 pM GM-CSF	50 pM GM-CSF	500 pM GM-CSF
Medium	46 ± 4	35 ± 3	39 ± 7	39 ± 3
GM-CSF (pM)				
5	75 ± 7*	64 ± 7	51 ± 4	42 ± 2
50	79 ± 7*	72 ± 2*	69 ± 8	54 ± 7
500	128 ± 7*	92 ± 5*	77 ± 1*	70 ± 2*

Different concentrations of GM-CSF were placed in the upper and/or lower compartments of the chemotaxis chamber. Results are expressed as the number of migrated HEC (mean ± s.d. of three samples for each experimental point).

* $P < 0.05$ versus medium control.

contaminant (that is, monocytes), might directly or indirectly account for the actions of G-CSF and GM-CSF on HEC. HEC cultures consisted of homogeneous cells with typical cobblestone morphology, having (>99%) granular deposits of factor VIII antigen, as well as H-lectin antigen and class I, but not class II, histocompatibility antigens on the surface. We could not detect cells expressing CD11b, CD14 or CD3 with flow cytometry. Treatment with anti-CD11b or anti-CD3 and complement did not affect the responses of HEC to the two CSF. The human endothelial cell line EAhy926 (refs 17–19) migrated and expressed c-*fos* mRNA after stimulation with G-CSF and GM-CSF and had specific receptors for these molecules (data not shown). Collectively, these data showed that HEC do indeed have receptors for and respond to G-CSF and GM-CSF.

In checkerboard experiments[6], maximal induction of migration across filters occurred in the presence of a positive concentration gradient between the lower and upper compartments of the chamber (Table 2). With equal concentrations of GM-CSF above and below the filter, a smaller, but significant enhancement of HEC migration was observed, suggesting that there is a chemokinetic component, in addition to chemotaxis, in the action of GM-CSF on HEC locomotion. G-CSF and GM-CSF also induced migration when HEC had adhered to the filters before the assay, excluding the possibility that these cytokines were merely affecting the speed of adhesion of HEC to filters.

The results presented here demonstrate that G-CSF and GM-CSF induce migration and proliferation of HEC, in what we believe is the first demonstration of activity of these molecules on non-haematopoietic cells. Other examples, of leukocyte-specific non-pleiotropic cytokines acting on non-hematopoietic elements, are monocytic-CSF acting on trophoblast cells and IL-2 acting on glial cells[20,21]. Our observation is interesting in the light of the many similarities between myelomonocytic cells and endothelium, illustrated by previously widespread use of the expression 'reticuloendothelial system'.

G-CSF and GM-CSF are produced by various cell types including stimulated lymphocytes, activated macrophages[1,2] and endothelial cells exposed to monokines[22,23]. Il-1 has been reported to induce proliferation of vascular elements[24] and c-*fos* expression in HEC[25]. The functionally related monokine tumour necrosis factor, however induces the migration of endothelial cells[26], but inhibits their proliferation[27]. These monokines do, however, elicit a complex set of responses in HEC, which include CSF production. For example, IL-1-induced proliferation of vascular smooth muscle is masked by the concomitant induction of growth inhibitory prostaglandins[24]. Proliferation of endothelial cells and neovascularization occur at sites of cell-mediated immunity and inflammation[28] and locally produced G-CSF and GM-CSF may be one of the factors involved in the fine regulation of the proliferation and migration of endothelium. Survival and growth of endothelium in bone marrow stroma is essential for the haematopoietic microenvironment and haematopoiesis. The capacity of appropriately stimulated HEC to produce G-CSF and GM-CSF suggests that these molecules may participate in autocrine regulation of function.

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Peptidyl-prolyl *cis-trans* isomerase is the cyclosporin A-binding protein cyclophilin

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Peptidyl-prolyl *cis-trans* isomerase (PPIase) catalyses the *cis-trans* isomerization of proline imidic peptide bonds in oligopeptides and has been shown to accelerate the refolding of several proteins *in vitro*[1–4]. Its activity has been detected in yeast, insects and *Escherichia coli* as well as in mammals, and it is thought to be essential for protein folding during protein synthesis in the cell[1]. We purified PPIase from pig kidney and found that its amino-acid sequence is identical to that reported for bovine cyclophilin, a protein known to bind the immunosuppressive drug, cyclosporin A (ref. 5). To investigate the functional relationship between

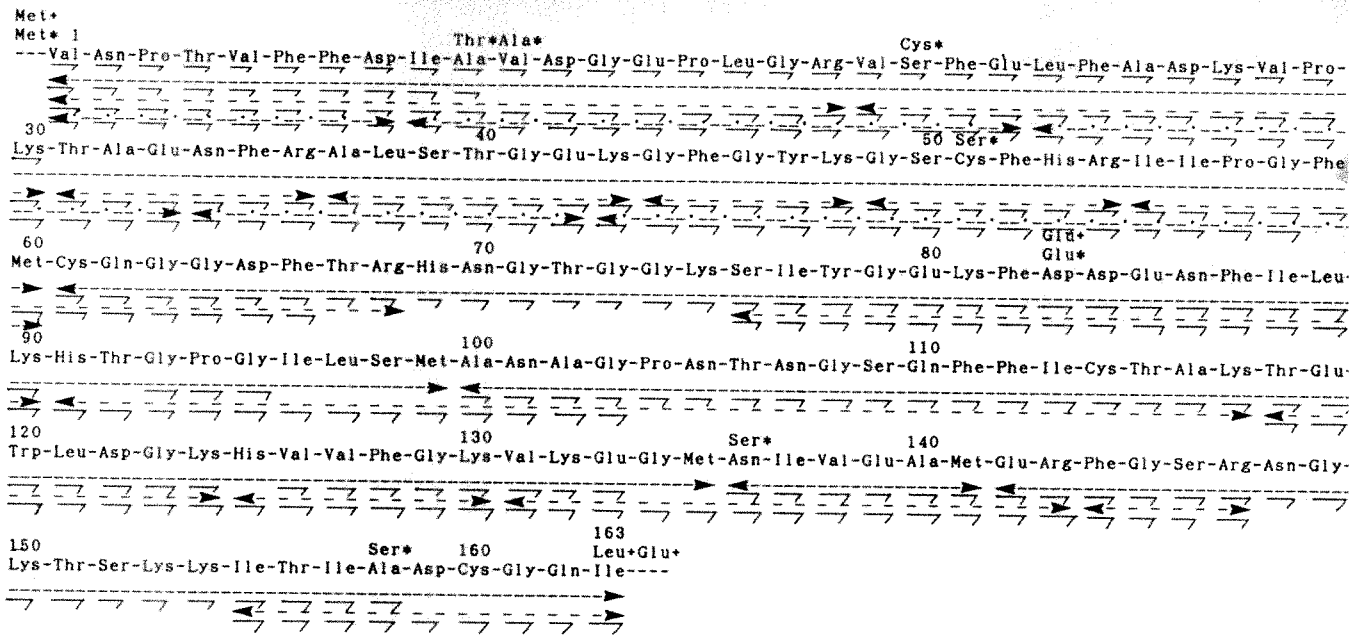


Fig. 1 Amino-acid sequence of form *a* of pig PPIase. The peptides from which the sequence was derived are as follow: - - -, tryptic peptides; - - - -, cyanogen bromide peptides; and —·—·—, *Staphylococcus aureus* V8 protease peptides of the amino-terminal cyanogen bromide peptide. After digestion of the native protein with either trypsin or cyanogen bromide, the peptides containing Cys 61 and Cys 160 purified as single peaks which could be separated further (to give the tryptic peptides 55–68 and 155–163, or the cyanogen bromide peptides 61–99 and 142–163) on reduction and carboxymethylation, indicating the existence of a disulphide bond. In contrast the peptides containing Cys 51 and Cys 114 purified separately, even without alkylation and reduction. The arrows at the amino terminus and under each peptide line indicate sequenator analyses of the intact form *a* and the peptides obtained. Differences between the pig and the rat, and/or the human sequences are indicated above the corresponding residues and designated by * for rat and by + for human. The amino-acid sequences of rat and human were deduced from their cDNA sequences[7,8].

Methods. The amino-acid sequence analysis was carried out by automated Edman degradation using Applied Biosystems 477A protein sequencer equipped with a model 120A on-line PTH analyser. The native protein (20–100 μg) was subjected to separate digestions with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin, and cyanogen bromide. The digests were separated on an Aquapore RP-300 column or on a Spheri-5RP-18 column with the use of 130A separation system (Applied Biosystems). The columns were eluted at a flow rate of 200 μl min^{-1} with a linear gradient of acetonitrile (0–100%) containing 0.1% trifluoroacetic acid or 0.1% heptafluorobutylic acid for 45 min. The amino-terminal cyanogen bromide peptide was further digested with *Staphylococcus aureus* V8 protease. Amino-acid analysis was carried out by Beckman 6300 E amino-acid analyser after hydrolysis with 6 M HCl for 24 h.

PPIase and cyclophilin we examined the effect of cyclosporin A on PPIase activity and found that it was inhibitory. Thus we propose that the peptidyl-prolyl *cis-trans* isomerizing activity of PPIase may be involved in events, such as those occurring early in T-cell activation, that are suppressed by cyclosporin A.

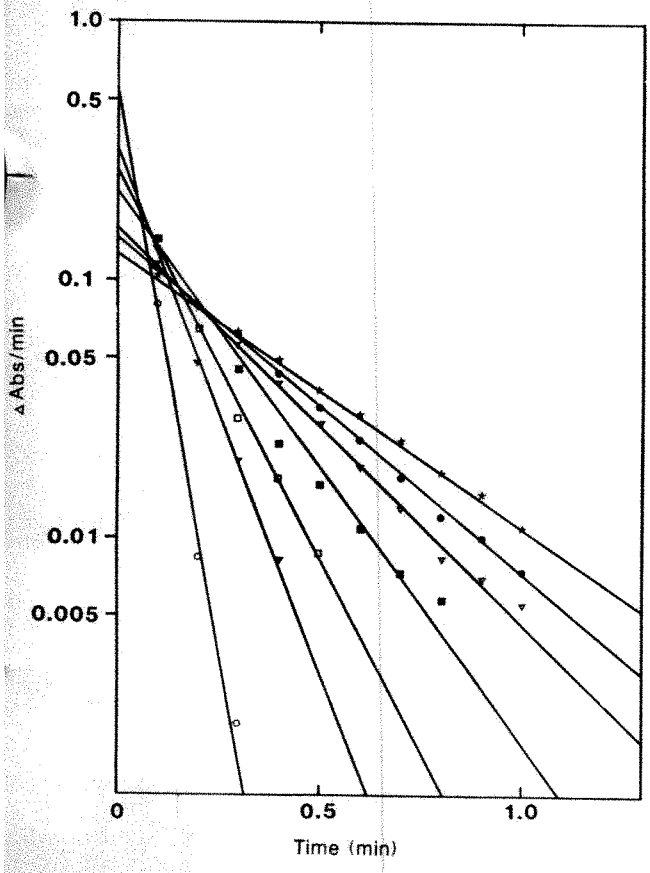
We detected PPIase activity, by the assay described in the Fig. 2 legend, in a crude ammonium sulphate extract prepared from pig kidney[1]. PPIase was purified further as described in Table 1, and after a final step of purification by reversed-phase column chromatography, was separated into two forms (*a* and *b*). These isoforms have an apparently identical relative molecular mass (M_r) of 18,000 and very similar amino-acid compositions (data not shown). Although the observed relative molecular mass is somewhat at variance with the value previously reported ($M_r = 14,000$)[3] we were not able to detect any other major component with PPIase activity during the purification. Exposure to a high concentration of acetonitrile during the reversed-phase column chromatography decreased but did not eliminate the specific activity detected (Table 1).

The two isoforms *a* and *b* were present in a ratio of 53:47 and their specific PPIase activities were indistinguishable. A comparison of their tryptic peptide maps indicated that the structural difference between the two isoforms resides in the amino-terminal peptides (data not shown). The first 30 amino-acid residues of form *a* were unambiguously determined by Edman degradation (see Fig. 1) but the amino terminus of form *b* could not be sequenced using this method.

Sequence analysis of form *a* reveals a total of 163 amino-acid residues and predicts a relative molecular mass of 17,735, in good agreement with the estimate by SDS-gel electrophoresis. Peptide mapping data indicate the existence of a disulphide bridge between Cys 61 and Cys 160, although we cannot exclude the possibility that this was formed *in vitro*, as we did not use anti-oxidant during the purification procedure. The remaining two cysteine residues, Cys 51 and Cys 114 (see Fig. 1 legend) appear to have free sulphydryl groups.

On searching for homologous sequences in the National Biomedical Research Foundation database, we found that the sequence of pig PPIase is identical to that of bovine cyclophilin. Cyclophilin is known to bind specifically to cyclosporin A (CsA), a cyclic undecapeptide of fungal origin, that is a potent immunosuppressant used to prevent rejection on transplantation of organs such as kidney, heart, bone marrow and liver[5,6]. Cyclophilin is a soluble cytosolic protein and is present in high concentrations (0.1–0.4% of total cytosolic proteins) in many mammalian tissues[5,6] and in nearly all organisms[5–7]. Two isoforms have been isolated for bovine cyclophilin and are expected to have very similar molecular structures[6].

The amino-acid sequence of pig PPIase (and bovine cyclophilin) is 96% identical to the published sequences for rat[7] and human[8] cyclophilin, and about 60% identical to the recently published sequence for *Neurospora crassa*[9], indicating a high degree of conservation across species. PPIase was suggested to be present on ribosomal particles[1] and it is interesting that



◀ **Fig. 2** Inhibition of PPIase activity by CsA. The kinetics of *cis-trans* isomerization of *N*-succinyl-Ala-Ala-Pro-Phe-MCA was monitored at 360 nm in a coupled assay with chymotryptic cleavage. The MCA moiety can only be cleaved off by chymotrypsin when the preceding Ala-Pro peptide bond is in the *trans* conformation[1]. At the equilibrium about 12% of the peptide has a *cis* Ala-Pro bond. In the presence of a high chymotrypsin concentration, hydrolysis of the *trans* peptide occurs within a few seconds, but cleavage of the *cis* peptide is limited in rate by the *cis* → *trans* isomerization of the Ala-Pro bond. The *trans* peptide was cleaved within the deadtime and the kinetic data of this phase is not shown. The rate of *cis* → *trans* isomerization of *N*-succinyl-Ala-Ala-Pro-Phe-MCA, represented as the absorbance change at a given time (ΔAbs/min) is plotted against the proceeding times (min) in semi-logarithmic plots. CsA clearly affected the rate of the isomerization catalysed by PPIase: ○, without CsA; ▼, 0.04 μg; □, 0.08 μg; ■, 0.16 μg; ▽, 0.4 μg; ●, 1 μg and ★, without PPIase.
Methods. Aliquots (2.0 ml) of 0.035 M HEPES-buffer containing 5 mM 2-mercaptoethanol, pH 7.8, were incubated at 25 °C for 10 min in the spectrophotometer cell (Spectrophotometer U-3210 with temperature controller SPR-7 and cell stirrer, Hitachi) before adding 50 μl 1.68 mM *N*-succinyl-Ala-Ala-Pro-Phe-MCA in the same buffer. Samples were equilibrated for 1 min. The PPIase fraction (0.27 μg) was incubated with the substrate for 30 s and the reaction was started by adding 20 μl 0.76 mM of chymotrypsin (sigma). The effect of CsA on PPIase activity was examined by mixing CsA with PPIase for 1 min before incubating with the substrate. CsA was kindly provided by Dr Y. Yamakawa of the National Institute of Health, Japan and was dissolved in saline containing 7% ethanol and 2.85% Tween 80 before use (this solvent did not affect the PPIase activity measured in these experiments). The rate of isomerization at a given time was calculated by using a rate assay program provided for Spectrophotometer U-3210.

Table 1 Purification of pig kidney PPIase

Purification step	Total protein (mg)	Total activity (arbitrary units)	Specific activity (arbitrary units mg^{-1})	Activity recovery (%)
Ammonium sulphate fraction	7,322	183,050	25	100
DEAE-Sephacel chromatography	699	90,222	129	49
Sephacryl S-200 gel filtration	67	63,784	952	35
Sephadex G-75 gel filtration	6.7	37,218	5,555	20
TSK-phenyl 5PW-RP chromatography				
form *a*	1.4	1,417	1,012	1
form *b*	1.2	1,368	1,146	1

The ammonium sulphate extract containing PPIase activity was prepared from 690 g of pig kidney cortex by the method of Fischer *et al.*[1]. The extract was dialysed against 0.01 M Tris-HCl buffer, pH 8.0, containing 0.05% NaN_3 and applied to a DEAE-Sephacel column (2.5 cm ID × 35 cm). PPIase activity was measured in a coupled assay with chymotrypsin by a modified method of Fischer *et al.*[1] in which the oligopeptide *N*-succinyl-Ala-Ala-Pro-Phe-4-methyl-coumaryl-7-amide (MCA) (Peptide Institute Inc., Osaka, Japan) was used as the substrate instead of *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide[4] or glutaryl-Ala-Ala-Pro-Phe-4-nitroanilide[1]. The eluate rich in PPIase activity passed through the column and was obtained as a single peak, which was concentrated by ammonium sulphate fractionation and separated on a Sephacryl S-200 column (2.5 cm ID × 90 cm) equilibrated with 0.1 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl and 0.05% NaN_3. A major PPIase activity was found in the last peak and this fraction was rechromatographed on a Sephadex G-75 column (2.5 cm ID × 90 cm). Finally, PPIase was purified by reversed-phase chromatography on a TSK-phenyl 5PW-RP column with a linear gradient of acetonitrile (0–70%) containing 0.1% TFA, and eluted as two peaks at about 40% of acetonitrile concentration. Although the specific activity of PPIase was decreased by one-fifth after the reversed-phase chromatography, it was associated with only the peaks of $M_r = 18{,}000$.

cyclophilin was found to be localized in both the mitochondria and the cytosol of *Neurospora crassa*[9].

The CsA-binding activity of cyclophilin is sulphhydryl dependent[5] and the activity of PPIase is also affected by sulphhydryl blocking reagents[1], suggesting that CsA binding may modulate PPIase activity. We therefore examined the effect of CsA and found that it blocked PPIase activity (Fig. 2). Activity was almost completely inhibited in the presence of CsA at 3×10^{-7} M, a concentration that is consistent with the dissociation constant of CsA to cyclophilin (2×10^{-7} M; ref. 5). These results, and the observation that both PPIase activity and the CsA-binding activity of cyclophilin are heat-sensitive at around 50 °C, and abolished by incubation with trypsin but not with chymotrypsin[1,6], support, on functional grounds, the identity between PPIase and cyclophilin.

On the basis of this identity, we propose that CsA may mediate some of its effects, such as the inhibition of interleukin-2 and γ-interferon production during T-lymphocyte activation, via its inhibitory action on PPIase. We also propose that PPIase may act, not only as an enzyme that facilitates folding during protein synthesis, but as a fundamental modulator in various intracellular signal transduction processes, though *cis-trans* isomerization of the partner molecules. The ubiquitious presence of PPIase, coupled with what we know of the effects of CsA-binding, may be indicative of its significance in such biochemical processes.

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Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins

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The enzyme peptidyl-prolyl *cis-trans* isomerase (PPIase) was recently discovered in mammalian tissues and purified from porcine kidney[1]. It catalyses the slow *cis-trans* isomerization of proline peptide (Xaa-Pro) bonds in oligopeptides and accelerates slow, rate-limiting steps in the folding of several proteins[2-5]. Here, we report the N-terminal sequence of PPIase together with further chemical and enzymatic properties. The results indicate that this enzyme is probably identical to cyclophilin, a recently discovered mammalian protein which binds tightly to cyclosporin A (CsA). Cyclophilin is thought to be linked to the immunosuppressive action of CsA[6-11]. The first 38 amino-acid residues of porcine PPIase and of bovine cyclophilin are identical and the two proteins both have a relative molecular mass of about 17,000 (ref. 7). The catalysis of prolyl isomerization in oligopeptides and of protein folding by PPIase are strongly inhibited in the presence of low levels of CsA. The activities of both PPIase and cyclophilin depend on a single sulphydryl group. At present it is unknown whether the inhibition of prolyl isomerase activity is related with the immunosuppressive action of CsA.

PPIase from pig kidney was subjected to automated N-terminal sequence analysis. The first 38 amino-acid residues of porcine PPIase are shown in Table 1. The sequence is identical to the N-terminal sequences of cyclophilin from human spleen and from bovine thymus[7,10]. An analysis[12,13] of the fluorescence and of the absorbance spectra of PPIase yields a content of one tryptophan and two tyrosine residues per PPIase molecule, which agrees with the respective values found for the cyclophilins[7,10]. Porcine PPIase and bovine cyclophilin[7] show the same apparent relative molecular mass (M_r) of 17,000 (17K). From the sequence analysis the actual M_r is 17,737, corresponding to 163 residues[7].

Cyclophilin forms very tight stoichiometric complexes with CsA. Values in the range of 5-200 nM were estimated for the respective dissociation constants[6,7]. CsA binds efficiently to PPIase as well. In the presence of CsA both catalysis of *cis-trans* isomerization of a proline-containing oligopeptide and the acceleration of protein folding are inhibited. The inhibition of the activity towards the peptide Suc-Ala-Ala-Pro-Phe-4-nitroanilide is shown in Fig. 1*a*. The rate of *cis* → *trans* isomerization of the Ala-Pro bond is increased from $k = 7.4 \times 10^{-3}\ s^{-1}$ (in the absence of PPIase) to $k > 1\ s^{-1}$ in the presence of 0.19 μM PPIase. Addition of 7 μM CsA abolishes the accelerating effect of PPIase and decreases the reaction rate to a value ($k = 9.0 \times 10^{-3}\ s^{-1}$) that is similar to the uncatalysed isomerization. The interaction is reversible: diluting out CsA by 2,000-fold restores PPIase activity. Figure 1*b* shows the decrease of PPIase activity with increasing concentrations of CsA. The resulting inhibition constant ($K_i = 2.6$ nM) agrees fairly well with the lower limit given for the dissociation constant ($K_d = 5$-200 nM) of the cyclophilin/CsA complex determined under different conditions[7].

The catalysis of slow protein folding reactions by PPIase is inhibited in the presence of CsA as well (Fig. 2). Slow refolding of ribonuclease T1 is strongly accelerated in the presence of 0.35 μM PPIase. Addition of 1.0 μM CsA inhibits catalysis of folding completely and a >95% inhibition of the PPIase activity towards folding of RNase T1 is found already in the presence of 0.5 μM CsA.

Both the binding of CsA to cyclophilin[8] and the catalysis of proline isomerization by PPIase[1] are sulphydryl dependent. One sulphydryl group of PPIase is specifically protected against modification with *p*(hydroxymercuri)-benzoate (*p*HMB) when CsA is bound to the enzyme. In the absence of CsA the modification reaction is triphasic. After a fast reaction with *p*HMB two slow phases are observed in which two and one sulphydryl group per PPIase molecule, respectively, are modified (Fig. 3*a*). The slowest phase is not observed in the presence of CsA. Apparently binding of CsA protects the most

Table 1 Determination of the N-terminal sequence of PPIase from porcine kidney

Cycle no.	Amino acid	Amount* (pmol)	Cycle no.	Amino acid	Amount* (pmol)	Cycle no.	Amino acid	Amount* (pmol)
1	Val	596	14	Glu	269	27	Lys	170
2	Asn	442	15	Pro	208	28	Val	118
3	Pro	554	16	Leu	180	29	Pro	96
4	Thr	119	17	Gly	245	30	Leu	146
5	Val	421	18	Arg	107	31	Thr	37
6	Phe	400	19	Val	132	32	Ala	111
7	Phe	429	20	Ser	44	33	Glu	68
8	Asp	403	21	Phe	142	34	Asn	84
9	Ile	345	22	Glu	90	35	Phe	133
10	Ala	313	23	Leu	103	36	Arg	55
11	Val	268	24	Phe	196	37	Ala	100
12	Asp	323	25	Ala	159	38	Leu	72
13	Gly	305	26	Asp	164			

PPIase was isolated from pig kidney cortex. After a 40-60% ammonium sulphate fractionation the protein was applied on a Sephadex cation exchange column at *p*H 8.5 and eluted with a linear KCl gradient (0-0.4 M). This was followed by gel filtration over Sephadex G-75 and chromatography on phenyl Sepharose or Fractogel TSK AF blue. The procedure resulted in a 780-fold purification and yielded material that migrated as a single 17 K band on denaturing polyacrylamide gels under reducing conditions. The N-terminal sequence was determined by automated Edman degradation using a 477A sequencer and an on-line 120A PTH analyser (both from Applied Biosystems). In addition to the 17 K PPIase, additional active protein species can be isolated which differ in M_r and isoelectric point. They will not be discussed further here.

* Yield of PTH amino acids at each step of the sequence analysis.

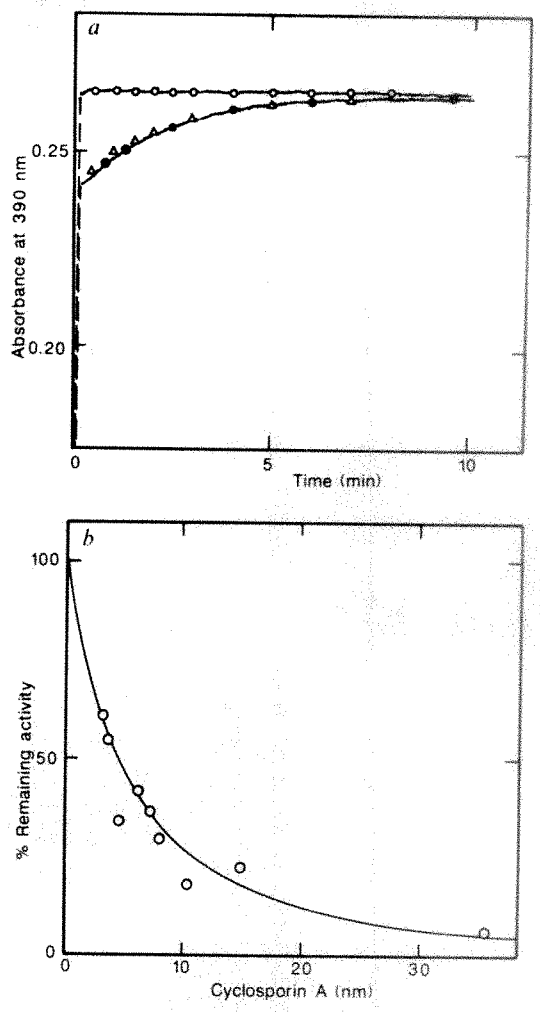


Fig. 1 Inhibition of PPIase by cyclosporin A. The activity of PPIase was determined by measuring the catalysis of the *cis* → *trans* interconversion of *cis* Suc-Ala-Ala-Pro-Phe-4-nitroanilide. *a*, *Cis* → *trans* isomerization (●) in the absence of PPIase ($k = 7.4 \times 10^{-3}\ s^{-1}$); (○) in the presence of 190 nM PPIase ($k > 1\ s^{-1}$); (△) in the presence of 190 nM PPIase + 7 μM CsA ($k = 9.0 \times 10^{-3}\ s^{-1}$). The *cis* content of the substrate remained unaffected by the presence of CsA. *b*, Dependence of residual PPIase activity on CsA concentration. 3.1 nM PPIase were preincubated in the presence of varying concentrations of CsA and the remaining PPIase activity was determined. The substrate concentration was much smaller than K_M, therefore no deviation from first-order kinetics were observed in the presence of the inhibitor CsA. An inhibition constant $K_i = 2.6 \pm 0.4$ nM can be calculated from the data.
Methods. The *cis* → *trans* isomerization of the Ala-Pro peptide bond in the test peptide N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide was measured in a coupled assay with chymotrypsin (Serva, Heidelberg), based on the ability of this protease to cleave the test peptide only when the Ala-Pro is *trans*[1,17]. α-Chymotrypsin (21 μM) was preincubated with the appropriate concentrations of PPIase and CsA and the assay was started by manual mixing with 30 μl of the test peptide in the spectrometer cell at 10 °C (the final concentration of test peptide was 22 μM). The *trans* peptide was cleaved within the deadtime; hydrolysis of the 4-nitroanilide in the *cis* peptide is limited in rate by the *cis* → *trans* isomerization at Ala-Pro and was followed by the increase in absorbance at 390 nm.

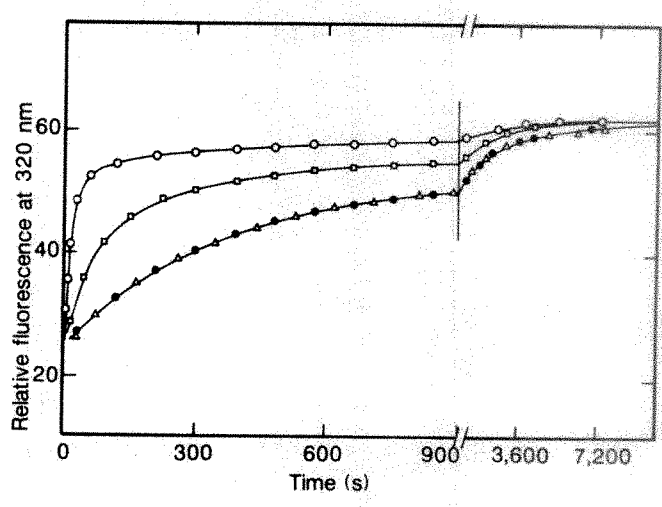


Fig. 2 Inhibition by CsA of the catalytic effect of PPIase on slow protein folding of RNase T1. The increase in fluorescence at 320 nm is shown as a function of the time of refolding in the absence of PPIase and CsA (●); in the presence of 0.35 μM PPIase (○); in the presence of 0.35 μM PPIase and 1.0 μM CsA (△); and in the presence of 0.35 μM PPIase and 0.5 μM CsA (□).
Methods. Recombinant RNase T1 was a gift from U. Hahn (Berlin). Unfolded protein was generated by a 2 h incubation of 13 μM RNase T1 in 8.0 M urea, 0.1 M Tris-HCl, *p*H 8.0 at 25 °C. Refolding was initiated by diluting 25 μl of the unfolded protein with 975 μl of 0.1 M Tris-HCl containing appropriate amounts of PPIase and CsA in the spectrophotometer cell at 10 °C. The final conditions for refolding were 0.33 μM RNase T1 in 0.2 M urea, 0.1 M Tris-HCl, *p*H 8.0 at 10 °C. Refolding was monitored by the increase in fluorescence at 320 nm (5 nm slit). Excitation was at 268 nm (5 nm slit). A Perkin-Elmer LS-5B fluorescence spectrophotometer was used.

owly reacting cysteine residue of PPIase against modification ith *p*HMB. Furthermore, completely SH-blocked PPIase no nger binds CsA, as could be shown in partition experiments f CsA between native PPIase and a large excess of the modified rotein. The inactivation by *p*HMB (Fig. 3*b*) and the odification of the least accessible cysteine (Fig. 3*a*) show very milar kinetics, indicating that this cysteine is directly involved the catalytic reaction of PPIase. In addition, binding of CsA hibits the irreversible inactivation by *p*HMB (Fig. 3*b*). A netic deuterium isotope effect, observed when α-C deuterated aa amino acids in -Xaa-Pro- containing peptides are used as substrates for PPIase[14], suggests that the substrate is covalently attached to the enzyme in the course of catalysis, probably as a hemithioorthoamide, which would allow free rotation about the C–N bond.

We conclude from these data that cyclophilin and PPIase are closely related or identical proteins. Both are widely distributed in different organisms and cell types[9]; they are abundant (cyclophilin 0.1–0.4%, PPIase 0.4% of total protein[7,15]), and they occur in different forms or isoenzymes (refs 6, 7 and unpublished observation by G. Fischer). The wide distribution of PPIase/cyclophilin and the highly conserved primary

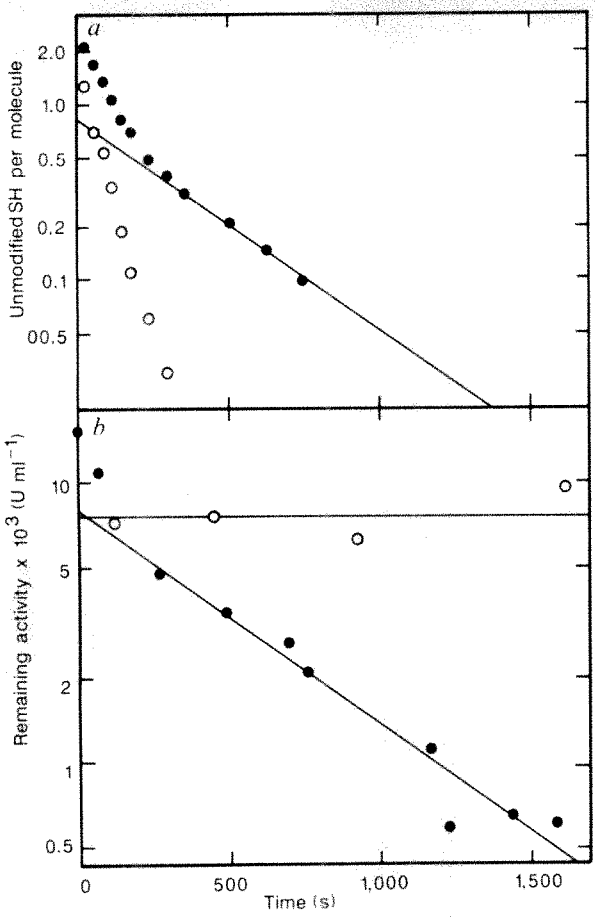


Fig. 3 Covalent modification of PPIase sulphydryls with *p*(hydroxymercuri)-benzoate in the absence (●) and in the presence (○) of 1.44 μM CsA. *a*, Time course of the modification of accessible thiols in 0.035 M HEPES buffer, *p*H 7.8 at 8.0 °C. PPIase (1.9 μM) was incubated with 28 μM *p*HMB and the extent of the modification reaction was followed by the increase in absorbance at 249 nm (with $\Delta\varepsilon_{249} = 7{,}000\ M^{-1}\ cm^{-1}$)[18]. A very fast modification reaction occurred within the deadtime of mixing, which reduced the concentration of *p*HMB to 16.1 μM. This burst may originate from the presence of rapidly reacting SH groups on the protein, but it probably results mainly from the presence of residual dithiothreitol in the enzyme solution. The slow part of the modification reaction was biphasic in the absence of CsA (●); 1.95 ± 0.2 SH groups per molecule reacted with $k_1 = 1.5 \times 10^{-2}\ s^{-1}$ and 0.8 ± 0.3 SH groups per molecule reacted with $k_2 = 2.8 \times 10^{-3}\ s^{-1}$. In the presence of CsA (○), 1.89 ± 0.2 SH groups per molecule were modified with a rate constant of $k = 1.55 \times 10^{-2}\ s^{-1}$. The slow phase was absent. *b*, Loss of catalytic activity of PPIase (1.9 μM) in the course of the reaction with *p*HMB in the absence (●) ($k = 2.0 \times 10^{-3}\ s^{-1}$) and in the presence (○) of 58.7 μM CsA. PPIase activity towards the test peptide was measured after a 1,200-fold dilution into the assay mixture. This high dilution was selected to decrease the concentrations of PPIase and of CsA below the K_i-value.

structure[7,10,11] suggest a fundamental role in cellular metabolism. Both CsA-binding and prolyl isomerase activity involve binding to a peptide chain and this activity could, therefore mediate binding to polypeptide chains in the cell, for example in the course of, or after, protein biosynthesis. Such a role would, however, be at variance with the proposed role of CsA in the regulation of gene transcription[16]. More extensive conclusions about the cellular function of PPIase/cyclophilin are not warranted because at present it is not known whether it is the actual target of CsA during immunosuppression and whether it is important for slow steps in the *in vivo* protein folding process.

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Aminoacylation of RNA minihelices with alanine

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The genetic code is determined by both the specificity of the triplet anticodon of tRNAs for codons in mRNAs and the specificity with which tRNAs are charged with amino acids. The latter depends on interactions between tRNAs and their charging enzymes, and an advance in understanding such interactions was provided recently by the demonstration that a major determinant of the identity of alanine tRNA is located in the amino-acid acceptor helix. Multiple substitutions in many distinct parts of the molecule do not prevent aminoacylation with alanine[1]. Substitution of the G3·U70 base pair with G3·C70 or A3·U70 in the acceptor helix prevents aminoacylation *in vivo* and *in vitro*, however[1], and the introduction of this base pair into $tRNA^{Cys}$ (ref. 1) or $tRNA^{Ph}$ (refs 1, 2) enables both to accept alanine. The importance of a single base pair in the acceptor helix and the results of recent footprinting experiments[3] prompted us to investigate the possibility that a minihelix, composed only of the amino-acid acceptor-TΨC helix, could be a substrate for alanine tRNA synthetase. We show here that a synthetic hairpin minihelix can be enzymatically aminoacylated with alanine. Alanine incorporation requires a single G·U base pair, and occurs in helices that otherwise differ significantly in sequence. Aminoacylation can be achieved with only seven base pairs in the helix.

Figure 1*a* shows the sequence and cloverleaf structure of $tRNA^{Ala/GGC}$, with the amino-acid acceptor-TΨC helix and loop indicated by shading. In the three-dimensional structure of tRNA, the four arms of the cloverleaf are combined into two helical segments which are approximately at right angles, forming an L-shaped structure. The amino-acid acceptor and TΨC sequences form one continuous helix that terminates at the 3′ end in four unpaired bases of the sequence $XCCA_{OH}$. In addition to the G3·U70 base pair, this structural motif of $tRNA^{A}$ has intrachain base pairing and stacking interactions, which promote stability. A significant amount of the synthetase-tRNA interaction is concentrated in the acceptor-TΨC helix, as indicated by the large block of contiguous phosphodiester bonds that are protected from nuclease digestion by the bound synthetase (Fig. 1*a*).

The resulting hairpin $minihelix^{Ala}$ (Fig. 1*b*) was synthesized using the template-primer system shown in Fig. 1*c*, and comprises twelve base pairs with a seven-nucleotide loop. The

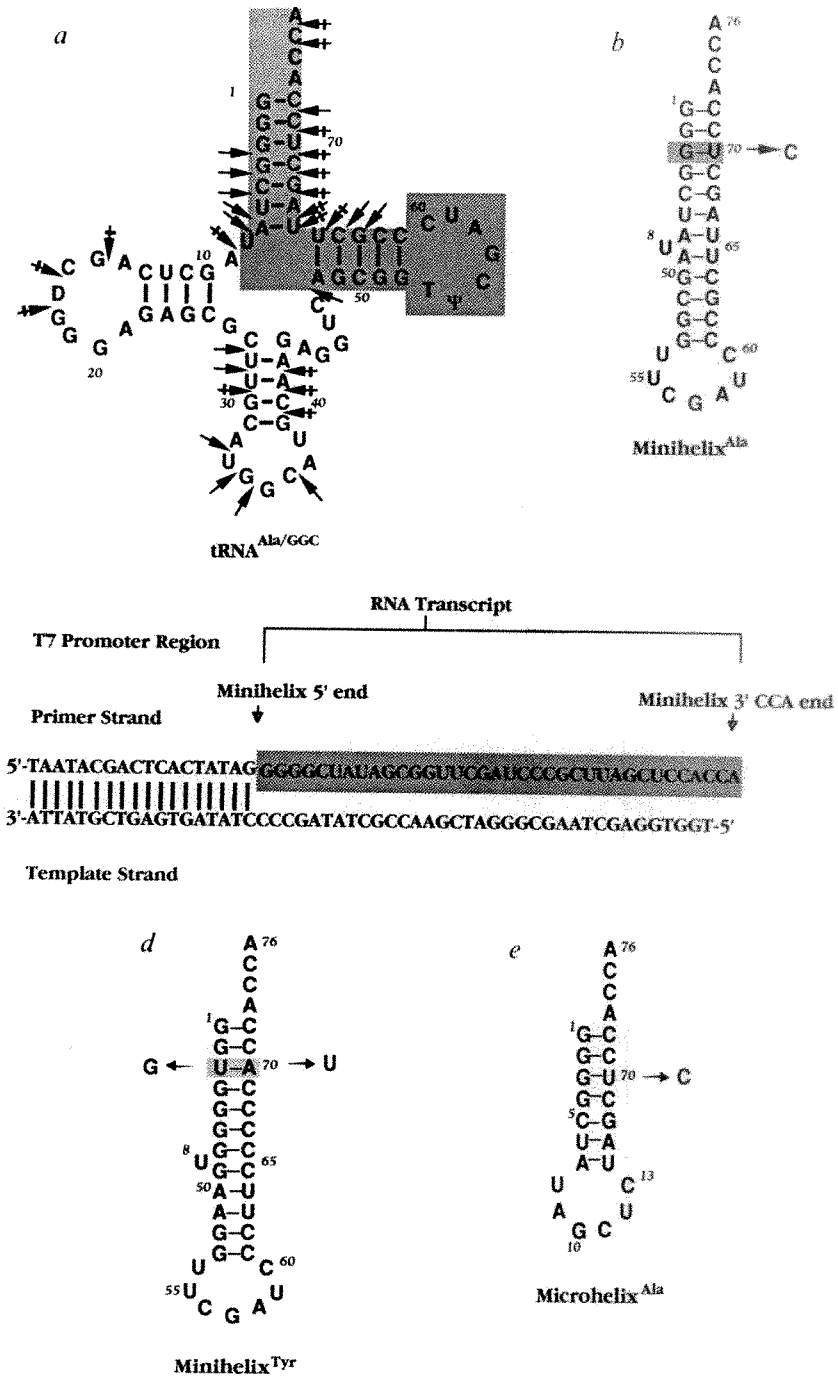


Fig. 1 *a*, Sequence and cloverleaf structure of $tRNA^{Ala/GGC}$ with nucleotides used in the construction of the minihelix indicated by shading. Phosphodiester bonds on $tRNA^{Ala/UGC}$ that have been probed by nucleases in the presence and absence of bound alanine tRNA synthetase are also shown (arrows)[3]. Those which are protected from nuclease attack are shown by cross-hatched arrows. *b*, Synthetic hairpin minihelix based on the acceptor-TΨC helix of $tRNA^{Ala/GGC}$. A variant of this helix substituted C70 for U70. Numbering is based upon that for intact $tRNA^{Ala}$. *c*, Template-primer system for the synthesis of minihelices and microhelices. The sequence of $minihelix^{Ala}$ is shown as an example. For the synthesis of other helices, a different DNA template strand was hybridized to the common 17-base pair T7 promoter strand. *d*, A synthetic minihelix based upon the acceptor-TΨC helix and loop of $tRNA^{Tyr}$. A second helix with a G3·U70 base pair was also made, as indicated. *e*, A synthetic microhelix based upon the acceptor helix of $tRNA^{Ala}$, with a loop comprised of nucleotides from U8 to C13.

Methods. The RNA minihelices and microhelices were synthesized in transcription reactions[22,23] using T7 RNA polymerase and a synthetic DNA template featuring a double-stranded promoter region and a long 5′ overhang corresponding to the transcribed sequence. T7 RNA polymerase was either purchased commercially (U.S. Biochemicals) or purified according to Grodberg and Dunn[24]. Transcription reactions were incubated for four hours at 37 °C in a buffer containing 40 mM Tris-HCl (*p*H 8.1), 5 mM dithiothreitol, 1 mM spermidine, 50 $\mu g\ ml^{-1}$ bovine serum albumin, 80 $mg\ ml^{-1}$ polyethylene glycol (relative molecular mass, M_r, of 8,000), 20 mM $MgCl_2$, 4 mM of each nucleotide, and 2.5 U μl^{-1} of T7 RNA polymerase. The DNA concentration in each strand was 0.25 μM. The reactions were terminated by phenol/chloroform extraction and then precipitated with ethanol. The full-length transcript was isolated by preparative gel electrophoresis from denaturing 20% polyacrylamide gels followed by electroelution or diffusion. The concentration of RNA in the preparations was determined by the absorbance at 260 nm at *p*H 7.5, 23 °C. A hyperchromicity value of 1.43 was used to correct the absorbance determinations. (This was obtained by measuring the absorbance of an RNA sample digested to completion with RNase T2 relative to an unhydrolysed sample.) For substrates that charged with alanine, the concentrations determined in this way (corrected when necessary for the minor fraction of molecules that terminated at their 3′-ends in C[25,26]), agreed with the concentrations determined by calculating the alanine acceptance.

uridine at position 8 was included in the acceptor-TΨC structure because this nucleotide is conserved in all prokaryotic and cytoplasmic tRNAs[4], is protected on one side by the bound enzyme (Fig. 1*a*), and is easily integrated into the acceptor-TΨC unit. We do not explore here whether this nucleotide is essential. Three additional minihelices were synthesized to test whether base substitutions in the acceptor stem exert the same effect on amino-acid acceptance that has been reported for the full-length RNA^{Ala} (ref. 1). A derivative of $minihelix^{Ala}$ was constructed in which U70 was replaced by C70 (Fig. 1*b*). This substitution has been shown to eliminate the aminoacylation function of RNA^{Ala} (ref. 1). A minihelix that corresponds in sequence to that of the acceptor-TΨC helix of $tRNA^{Tyr}$ was synthesized as well as a G3·U70 variant of that minihelix (Fig. 1*d*). The sequence of the acceptor-TΨC stem of $tRNA^{Tyr}$ differs from that of $tRNA^{Ala}$ at seven of 12 base pairs, providing a system in which to test whether G3·U70 retains its strong influence on alanine identity in a different sequence context. In addition to these four minihelices, a fifth helix (the 'microhelix') was synthesized. This consisted of the seven base pairs of the acceptor helix of $tRNA^{Ala}$, connected by a six base-pair loop (Fig. 1*e*). In the sequence of $microhelix^{Ala}$, the sequence of the loop starts at U8 and continues into the 5′ side of the D-stem, such that C13 is joined to U66. A G3·C70 variant of this sequence has also been constructed.

Aminoacylation of preparations of $minihelix^{Ala}$ was examined at pH 7.5 and 37 °C. As a negative control, wild-type $tRNA^{Tyr}$ was used as a substrate. At a concentration of 4.5 μM, the minihelix is aminoacylated at catalytic concentrations (15 nM) of alanine tRNA synthetase, whereas there is no detectable aminoacylation of the control under the same conditions (Fig. 2*a*). At higher concentrations of the enzyme (300 nM), near-complete aminoacylation of the minihelix is observed within 20 min (Fig. 2*a*, inset).

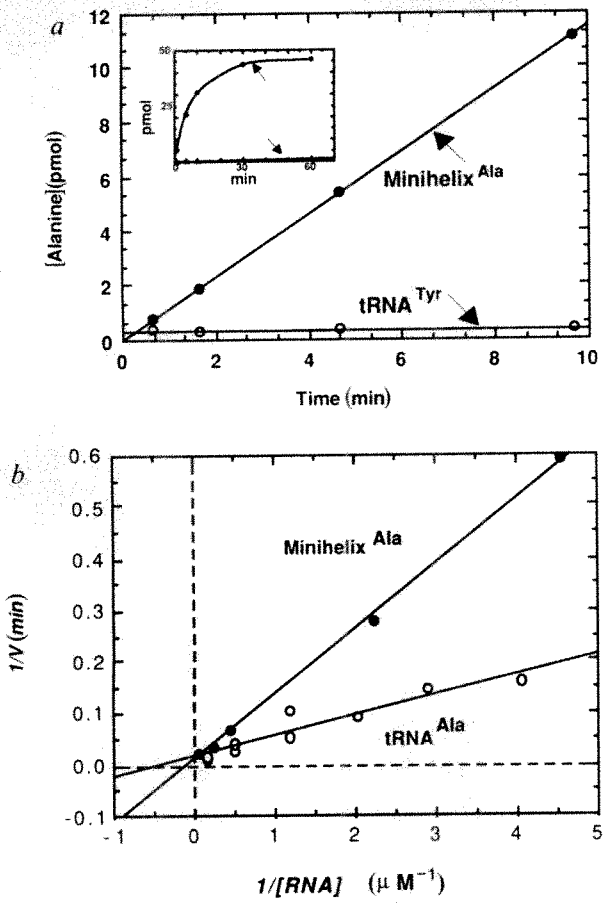


Fig. 2 *a*, Aminoacylation of $tRNA^{Tyr}$ and of $minihelix^{Ala}$. The assays were carried out at *p*H 7.5, 37 °C, as previously described[20,21] in a buffer containing 50 mM sodium phosphate (*p*H 7.5), 100 μg ml^{-1} bovine serum albumin, 20 mM potassium chloride, 10 mM $MgCl_2$, 20 mM β-mercaptoethanol, 4 mM ATP, 20 μM alanine, 2.4 μM [2,3-^{3}H] alanine and 4.5 μM $minihelix^{Ala}$ RNA or 5 μM $tRNA^{Tyr}$. The reactions were initiated by adding purified alanine tRNA synthetase to a final concentration of 15 nM. The inset shows an experiment that compares the aminoacylation of 2.5 μM $minihelix^{Ala}$ with 5 μM $tRNA^{Tyr}$ at an alanine tRNA synthetase concentration of 300 nM. *b*, Plot of inverse velocity against inverse RNA concentration for the aminoacylation of $tRNA^{Ala}$ and $minihelix^{Ala}$. Each point was obtained from the determination of the initial linear rate of aminoacylation at varying substrate concentrations of either the minihelix (over a ten-min interval) or of $tRNA^{Ala}$ (over a 2.5-min interval). The concentration of purified alanine-tRNA synthetase was 15 nM. The reaction conditions were otherwise similar to those in Fig. 2*a*.

A determination of the initial rates of aminoacylation at several substrate concentrations allowed the individual kinetic parameters, the Michaelis constant K_m, and k_{cat} to be determined separately for two substrates. Figure 2*b* shows a plot of inverse velocity against inverse RNA concentration. The plots for both $tRNA^{Ala}$ and $minihelix^{Ala}$ extrapolate to nearly the same intercept on the ordinate, indicating that the enzyme has a similar maximal velocity for the two substrates. The K_m parameter is about fivefold higher for the minihelix, however, suggesting that it binds less efficiently to the enzyme (Table 1). This factor of five corresponds to about one kcal mol^{-1}, which is sufficiently small to be accounted for by one or two van der Waals' contacts that may be absent in the enzyme-minihelix complex, as compared to the analogous complex with wild-type $tRNA^{Ala}$. Because k_{cat} and K_m are close to values observed for the native tRNA, the fragment must possess the structures required for both binding and passage through the transition state.

Table 1 Kinetic parameters for the aminoacylation of $tRNA^{Ala}$ and of helical fragments with *Escherichia coli* alanine tRNA synthetase

Substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
$tRNA^{Ala}$	2.0	0.89	4.4×10^5
$Minihelix^{Ala}$	11.4	1.5	1.3×10^5
G3·U70 $minihelix^{Tyr}$	8.8	0.48	5.3×10^4
$Microhelix^{Ala}$	35.9	0.28	7.7×10^3

Aminoacylation reactions were carried out as described in the Fig. 2 legend. The concentration ranges of each of the substrates used to measure the kinetic parameters were as follows: $tRNA^{Ala}$, 0.25–6.4 μM; $minihelix^{Ala}$ and G3·U70 $minihelix^{Tyr}$, 0.22–25 μM; and $microhelix^{Ala}$, 0.5–25 μM. Purified alanine tRNA synthetase was used at a concentration of 15 nM (as determined by active site titration[19,20]) for all kinetic assays. The parameters for all of the RNA substrates were determined at a saturating ATP concentration (4 mM) but at a subsaturating concentration (20 μM) of alanine. The high K_m of alanine tRNA synthetase for alanine (0.25 μM[20]) required prohibitively high radioactive alanine concentrations to achieve saturation. In earlier work, it has been shown that the K_m for $tRNA^{Ala}$ shows little sensitivity to the concentration of alanine[21].

The G3·C70 variant of $minihelix^{Ala}$ was synthesized and found to be completely inactive for aminoacylation with alanine, even under conditions where a relatively high (20-fold higher than that used for the kinetic parameter determination) enzyme to substrate ratio was used (Fig. 3*a*). This suggests that the G3·U70 base pair is also important in determining the amino-acid identity of the minihelix, as it does in the intact $tRNA^{Ala}$ (ref. 1).

A 'tyrosine' minihelix was also synthesized (Fig. 1*d*) and tested for aminoacylation with alanine. As in the case of $minihelix^{Ala}$, the presence of G3·U70 is required for efficient aminoacylation. The rate of aminoacylation of the G3·U70 variant of $minihelix^{Tyr}$ is only 2.5-fold slower than that of the $minihelix^{Ala}$. This result indicates that alanine tRNA synthetase does not exert significant catalytic discrimination against the six base pairs that are not common to $minihelix^{Ala}$ and G3·U70 $minihelix^{Tyr}$. On the basis of a determination of the kinetic parameters for G3·U70 $minihelix^{Tyr}$, the rate reduction can be largely attributed to the k_{cat} parameter (Table 1).

The amino-acid acceptor portion of $tRNA^{Ala}$ comprises only seven base pairs. We observed that a 'microhelix' based on just the sequence of the $tRNA^{Ala}$ acceptor stem can be completely aminoacylated by the enzyme, at a reduced rate relative to that of $minihelix^{Ala}$ (Fig. 3*b*). The kinetic parameters for $microhelix^{Ala}$ were determined under the same conditions as for the minihelices (Table 1). The K_m for $microhelix^{Ala}$ is 18-fold higher and the k_{cat} is threefold lower than the respective parameters for $tRNA^{Ala}$. The differences between the values for $microhelix^{Ala}$ and $minihelix^{Ala}$ demonstrate the effect of the removal of sequences corresponding to the TΨC stem and loop. The greater effect on K_m suggests that, in the progressive deletion of sequences outside the acceptor stem, some contacts with the enzyme have been lost. On the basis of the relative Michaelis constants (K_m) for $tRNA^{Ala}$ and $microhelix^{Ala}$, the decrease in binding energy is about 1.7 kcal mol^{-1}. The lost contacts do not seem to be crucial to catalysis however, because the effect on k_{cat} is relatively small. Other experiments showed that efficient aminoacylation of the microhelix requires the G3·U70 base pair (data not shown).

Although the influence of the G3·U70 base pair on the K_m parameter is important in discriminating $tRNA^{Ala}$ from other tRNAs, there is evidence that the k_{cat} parameter is more important[5]. For certain tRNAs, some or all of the sequences which determine identity are located in the anticodon[6–10]. The data in Table 1 show that for $microhelix^{Ala}$, the k_{cat} parameter is within a factor of three of that for wild-type $tRNA^{Ala}$. Thus, once bound

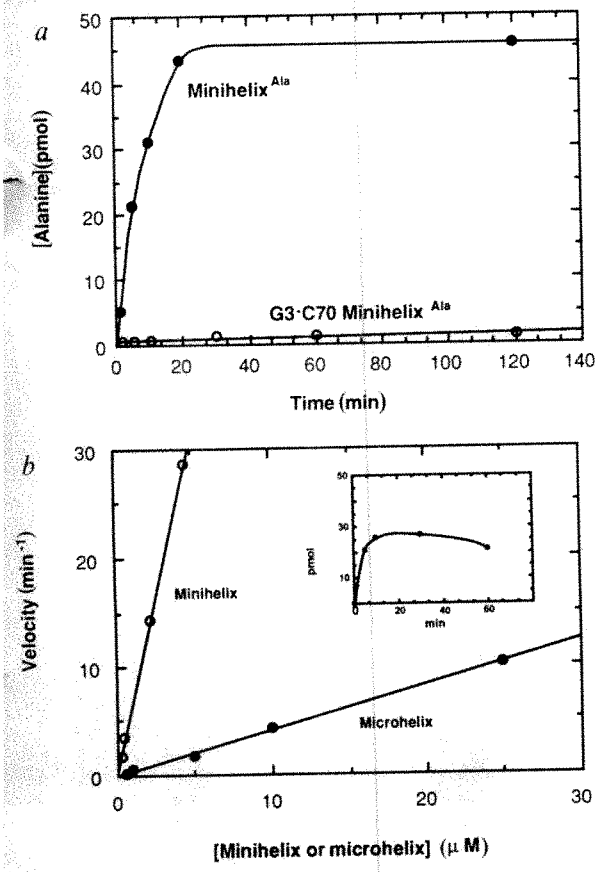


Fig. 3 *a*, Initial rate of aminoacylation with alanine of the 'wild-type' and G3·C70 variant of minihelix^{Ala}. The RNA substrates were present at a concentration of 5 μM, and alanine tRNA synthetase was added to a final concentration of 300 nM, which is a twentyfold increase over the concentrations used in the determination of kinetic parameters. *b*, Initial velocity of aminoacylation with alanine against the concentration of mini- and microhelix^{Ala}. The concentrations of the RNA substrates were as follows: minihelix^{Ala}, 0.25–4.5 μM; microhelix^{Ala}, 0.5–25 μM. Alanine tRNA synthetase was added to a final concentration of 15 nM, and the reaction conditions were similar to those in Fig. 2. The inset shows an aminoacylation experiment where the concentration of microhelix^{Ala} was 3 μM and the concentration of alanine tRNA synthetase was 1 μM.

to the enzyme, the microhelix is aminoacylated at a comparable rate to that of intact, native tRNA^{Ala}.

Although we have confirmed that G3·U70 acts as a positive determinant for recognition by alanine tRNA synthetase, we cannot rule out the possibility that tRNA^{Ala} possesses negative determinants that reduce its interactions with non-cognate synthetases. These negative determinants may, in part, lie outside the acceptor-TΨC helix.

Earlier studies suggested that partial tRNA molecules or tRNA fragment combinations might be substrates for aminoacylation *in vitro*, although the overall yields of aminoacylation were 5% or less in some cases[11,12]. There are examples of naturally-occurring tRNAs that are truncated versions of the typical four-armed cloverleaf pattern, although they are not as reduced in size as the molecules studied here. In the sequence analyses of several mammalian mitochondrial genomes, a tRNA$^{Ser/AGY}$ has been identified that has an ordered structure in which the D-stem and loop are absent[13,14]. Also, at least one other enzyme involved in tRNA metabolism (RNase P) is able to use RNA substrates that are smaller than a complete tRNA[15].

It has been speculated that, during an early stage of evolution, small tRNA molecules were esterified with amino acids in a system that was a precursor to protein synthesis (see summary in ref. 16). These molecules could have had sequences that influenced the selection of the amino acid attached by the primordial synthetase[17]. The data presented here show that, for one amino acid at least, information sufficient for its specific attachment to an RNA molecule can be encoded by sequences proximal to the 3′-amino-acid attachment site.

It has been speculated that primitive RNA genome molecules were tagged by tRNA-like structures in early evolution[18]. These tags might have been used by a primordial enzyme as replication initiation points. In the light of these suggestions, our results invite speculation that these tags could have been short RNA minihelices, which after serving as primitive RNA telomeres, were released to become pre-tRNAs upon which the first peptides were synthesized.

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Long-range structural changes in proteinase K triggered by calcium ion removal

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The X-ray crystal structure of the subtilisin-type enzyme proteinase K at 1.5 Å resolution[1] shows that is has two binding sites for Ca^{2+}. Scatchard analysis[2] indicates that one Ca^{2+} binds tightly, with pK 7.6×10^{-8} M^{-1}, and the other only weakly. Although Ca^{2+} is not directly involved in the catalytic mechanism and is 16.6 Å away from the α-carbon atoms of the catalytic triad Asp 39–His 69–Ser 224, the activity of proteinase K towards the synthetic substrate succinyl-Ala-Ala-Ala-*p*-nitroanilide drops slowly to ~20% of its original value when it is depleted of Ca^{2+}. This is not due to autolysis of the enzyme[2]. The X-ray crystal structure of Ca^{2+}-free proteinase K shows that removal of Ca^{2+} from the tight binding site triggers a concerted domino-like movement of five peripheral loops and of two α-helices. At a distance of 25 Å from this

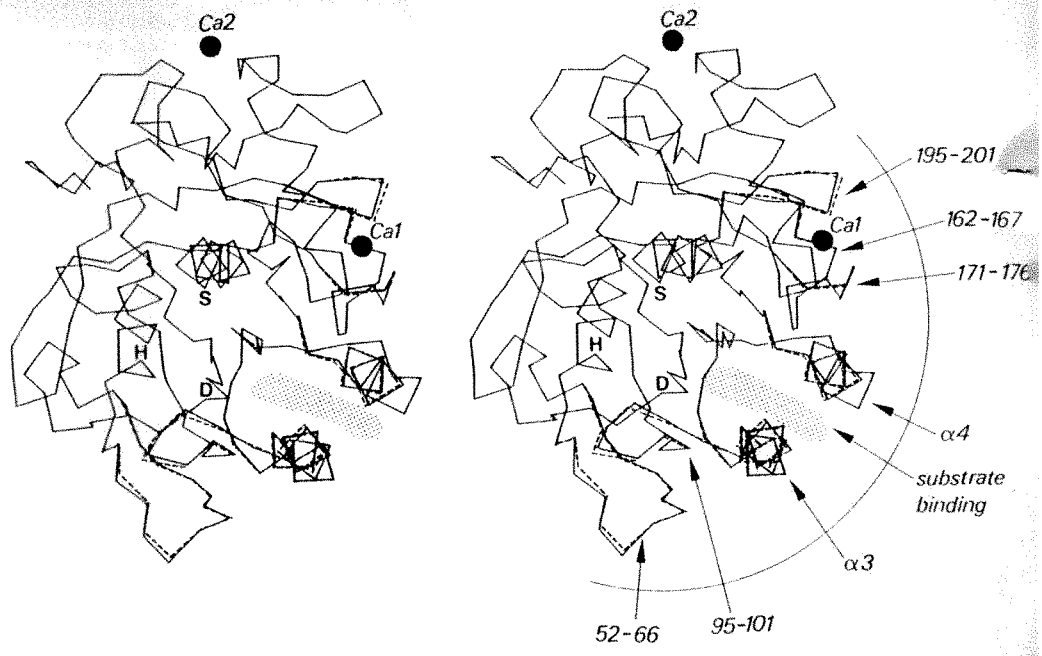


Fig. 1 Stereo view of C_α-superposition of Ca^{2+}-containing and Ca^{2+}-free proteinase K molecules. The least-squares fit procedure for the whole molecule gives an r.m.s. deviation of 0.22 Å. If only the 'isomorphous' atoms that are in identical positions within ~0.1 Å are included, the r.m.s. deviation is 0.04 Å. Sections where differences in atomic coordinates exceed 0.2 Å have an r.m.s. deviation of 0.74 Å and are shown in broken lines for Ca^{2+}-free proteinase K. The Ca^{2+}-binding sites Ca1 and Ca2, the substrate-binding site and the segments that move after removal of Ca^{2+} are indicated. Significant autolysis of Ca^{2+}-free proteinase K occurs only 48 h after incubation[1]; crystals were obtained after 12 to 24 h.

Methods. Proteinase K (Merck, Darmstadt) was dissolved to 10% concentration in 50 mM Tris-HCl, *p*H 7.8, 280 mM Na-EDTA. Vapour diffusion against 0.8 M $NaNO_3$ yielded crystals isomorphous with the native Ca^{2+}-containing enzyme, of space group $P4_32_12$, $a = b = 68.3$ Å, $c = 108.4$ Å. Film data to 2.18 Å resolution were collected on an Arndt-Wonacott camera (Elliott GX20 generator, CuK_α graphite monochromator); there were 11,634 independent reflections ($R_{merge,sym,I} = 5.2\%$) with 85% $>1\sigma_F$. Phasing was with a 1.5 Å 'native' model[1]; manual model building was based on $2F_o - F_c$ and 'omit' maps (FRODO[12]; Evans and Sutherland, PS 300), followed by restrained least squares refinement cycles[13]. *R*-factor is 18.1% for all data $>3\sigma_F$; model consists of C, N, O and S atoms and 94 water oxygens with $B < 30$ Å. Drawn with the program in ref. 14.

calcium-binding site, the geometry of both the secondary substrate binding site and of the catalytic triad is affected by this movement thereby reducing the activity of the enzyme.

The enzyme activity of proteinase K from the mould *Tritirachium album* Limber is so high that it will even digest keratin[3,4]; it is a calcium-dependent enzyme. The catalytic triad Asp 39-His 69-Ser 224 and the two strands of the substrate-binding region corresponding to residues 100–102 and 132–135, form an antiparallel triple β-sheet structure with the substrate, and have been characterized by crystal structure analysis of proteinase K bound to two different peptide chloromethane inhibitors[5,6]. The more strongly binding of the two Ca^{2+}-binding sites (Ca1) is well defined and coordinates in the form of a pentagonal bipyramid: if the two side-chain carboxyl oxygen atoms of Asp 200 are taken as one ligand, then the Pro 175 C=O and Asp 200 $O_{\delta1}O_{\delta2}$ are in apical positions, with Val 177 C=O and four water molecules occupying the equator. In contrast, the coordination sphere of the weaker binding site (Ca2) is incompletely defined in the X-ray structure, by Thr 16 C=O, Asp 260 $O_{\delta1}O_{\delta2}$ and two water molecules[1].

If these Ca^{2+} ions are removed using EDTA and subsequent gel filtration, the enzyme activity of proteinase K decreases by ~80%, falling with $t_{\frac{1}{2}} \sim 3$ h. Activity can be restored to only 28% by excess Ca^{2+} (ref. 2). This behaviour is not associated with autolysis of the enzyme, which becomes significant only after 48 h incubation[2], so we interpret it as resulting from structural changes induced when proteinase K is depleted of Ca^{2+}. We therefore undertook an X-ray crystallographic study of the Ca^{2+}-free enzyme; methods are given in the legend to Fig. 1.

Figure 1 shows that there are no cuts in the polypeptide chain and that the main portion of the molecular structure of proteinase K, including the weaker binding site Ca2, is only marginally affected by removal of Ca^{2+} if at all. Significant structural changes giving shifts greater than 0.2 Å in atomic coordinates occur in a coherent peripheral section. These are indicative of a concerted motion triggered by the substitution of $Ca^{2+} \cdot 4H_2O$ by two water molecules at the Ca1 binding site (Fig. 2); this motion propagates in a domino-like way through adjacent segments of the molecule. These segments involve the two peripheral α-helices, α3 and α4, and the five loops comprising residues 195–201, 162–167, 171–176, 95–101 and 52–66, which make contact through van der Waals interactions, hydrogen bonds and a salt bridge (Table 1). These subtle local changes in molecular structure are associated with more severe positional shifts, deletions and additions of water molecules in the first hydration sphere, suggesting that alterations in the peripheral tertiary hydrogen bonding are substantial. The motion is bounded in an anticlockwise sense (Fig. 1) by a loose connection between loop 195–201 and the protein carboxyl terminus, which acts as buffer, and in 'clockwise sense' by loop 52–66 which is 30 Å away from Ca1 and is exposed to solvent. The movement of the tyrosine residues in loop 52–66 was also detected by circular dichroism[2], suggesting that these structural changes that occur on removal of Ca^{2+} are an intrinsic property of the molecule, and not a result of crystal lattice effects.

The secondary substrate binding site is formed by two strands, from residues 100–102 and 132–135. The latter is linked with site Ca1 through the loop of amino acids 171–176. The shift expected after Ca^{2+} removal (accompanied by isomerization of *cis* Pro 171 to the *trans* form) with concomitant reduction in enzymatic activity[2], does not occur; probably because loop 171–176 is tightly anchored on to helix α6 by a disulphide bond from Cys 178–Cys 249 (Fig. 2a and b). Instead, the peptide carbonyl group of Gly 100, which is part of loop 95–101 and 25 Å away from Ca1, is shifted by ~0.8 Å, so that hydrogen bonding to the substrate is diminished or even impeded (Fig. 2*c*). Side chains of catalytic triad sequence Asp 39–His 69–Ser 224 are rotated somewhat. Consequently, the hydrogen bond between Ser 224 O_γ and $N_{\varepsilon2}$ His 69, which is necessary for the catalytic proton transfer, is lengthened from 3.01 Å in the 'native' Ca^{2+}-bound enzyme to 3.54 Å in the Ca^{2+}-free state (Fig. 2*d*).

The shifts in atomic positions at the catalytic centre can explain the reduction in enzymatic activity of proteinase K on removal of Ca^{2+}. That the activity is not lost completely is

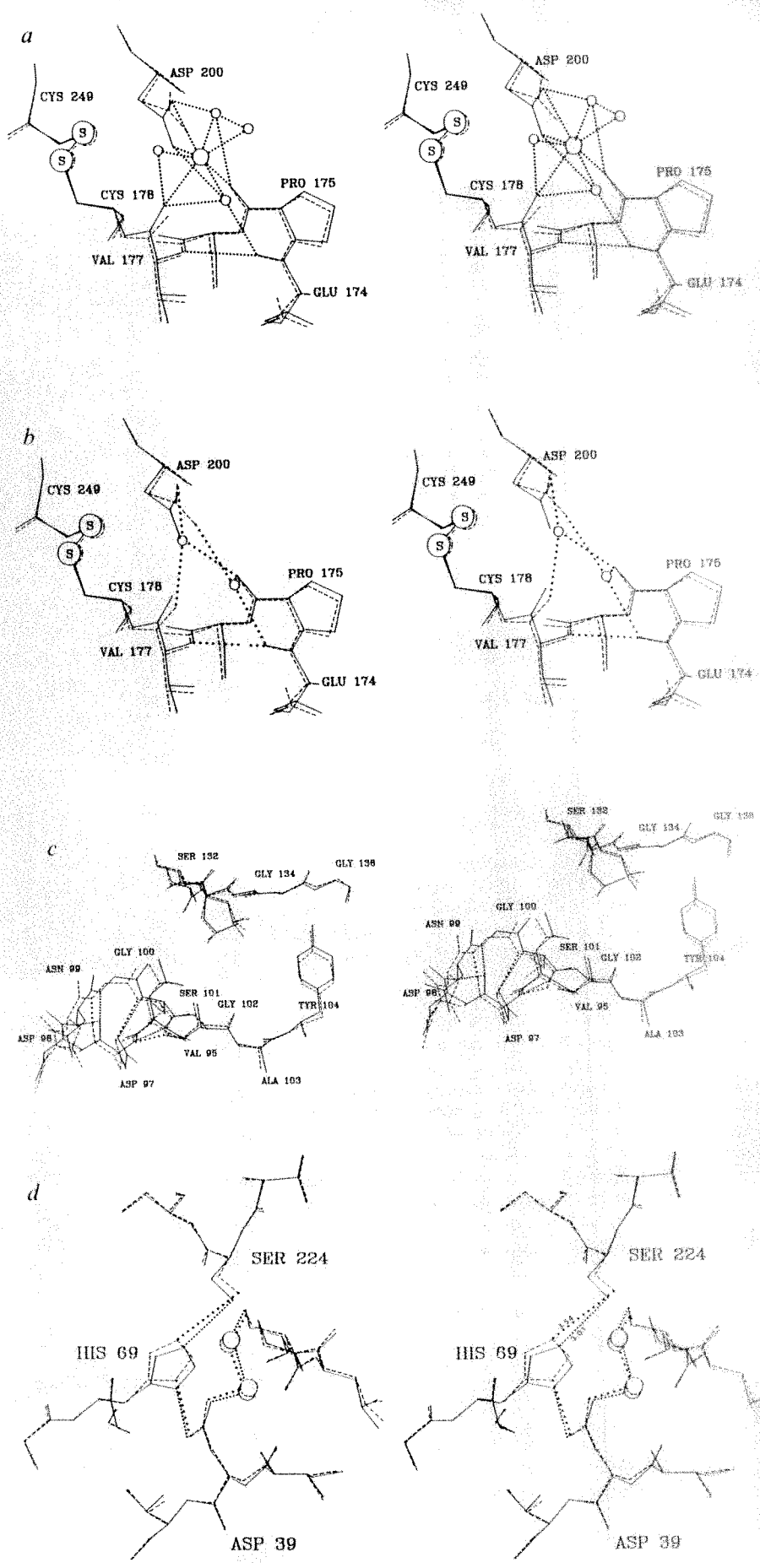


Fig. 2 *a–d*, Stereo views of different sections of the molecular structure of 'native' proteinase K containing Ca^{2+} (solid lines) and of Ca^{2+}-free proteinase K (broken lines). Hydrogen bonds and Ca^{2+} coordination bonds are indicated by dotted lines, the smaller dots referring to Ca^{2+}-containing proteinase K and larger dots to Ca^{2+}-free proteinase K. Drawn with the program from ref. 14. *a*, *b*, The Ca^{2+}-binding site Ca1 drawn with (*a*) and without (*b*) Ca^{2+}. Coordination and hydrogen bonds indicated by dotted lines, small spheres are water molecules, the larger sphere is Ca^{2+}. The disulphide bond is marked S; Cys 249 is attached to helix α6. The secondary substrate-binding site (segment 130–134) that does not shift significantly when Ca^{2+} is removed is at the lower left (not shown). *c*, The secondary substrate-binding site. The structure of segment 132–135 is almost unaffected by removal of Ca^{2+}, whereas the segment 100–102 changes position and conformation. The hydrogen bonds with Asp 97 (dotted lines) are all lengthened in the Ca^{2+}-free enzyme by 0.3–0.5 Å. *d*, View of the catalytic site. Main-chain atoms are almost unaffected, but side chains shift so that the length of the critical hydrogen bond between Ser 224 O_γ and $N_{\varepsilon 2}$ His 69 increases by 0.53 Å when Ca^{2+} is removed. Water molecules are drawn as spheres.

Table 1 Intramolecular interactions between segments which move domino-like when Ca^{2+} is removed from proteinase K

Segment*	Interactions
195–201	Ala 164 C=O···Tyr 195 N
	Ala 166 N···Tyr 196 C=O
162–167	Asp 165 O_δ···W326···Ser 197 O_γ
	Asp 200 C ($O_{\delta1}$···W283; $O_{\delta2}$···W282) ⊖ — Val 177 C=O; Glu 174 C=O; Pro 175 C=O
171–176	
	Asn 142 $N_{\delta1}$···Ser 173 C=O
	Ala 146 C=O···W317···Ser 176 C=O
142–154 (α4)	Asp 112 C ($O_{\delta1}$···$N_{\eta1}$; $O_{\delta2}$···$N_{\eta2}$) ⊖ ⊕ C Arg 147
95–101	Met 111≡(Ala 144, Leu 148)
and α3	(Trp 91, Val 95, Leu 96)≡(Tyr 59, Tyr 60, Tyr 61)
	Asp 98 C=O···$N_{\delta2}$; $O_{\delta1}$···His 69 N; C
52–66	(active site)
	Asn 67

Dashed lines represent hydrogen bonds X—H···A, with 2.68 Å ≤ X···A ≤ 3.20 Å; triple lines represent hydrophobic interactions. The two peripheral α-helices involved in the movement are α3 (residues 103 to 121) and α4 (residues 142 to 154).

*See Fig. 1.

probably due to the relatively subtle nature of the structural changes. The slow rate of this reduction in activity can be explained by the tightly bound Ca^{2+} at site Ca1 resisting chelation by EDTA, together with the associated movements in the hydration sphere which probably dampen this process even further. The altered hydration of Ca^{2+}-free proteinase K could trap the enzyme in a new energy minimum (calculations to be published). This would explain why the activity of proteinase K is not fully recovered after addition of excess Ca^{2+} (ref. 2).

Proteinase K does not autolyse after removal of Ca^{2+} for 48 hours (ref. 2), probably because the disulphide bridge Cys 178–Cys 249 stabilizes the geometry of the 'empty' Ca1 binding site, thereby preventing even partial unfolding. Two related subtilisins, BPN and Novo, also have two Ca^{2+} binding sites, but in different positions[7–11], and they do not contain disulphide bridges. This could explain why they unfold after depletion of Ca^{2+} and become sensitive to enzyme attack to autolyse readily.

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Advances in two-dimensional electrophoresis

S.M. Hanash and J.R. Strahler

New developments in the first dimension step of two-dimensional electrophoresis have expanded the utility of the technique in cell and molecular biology.

WHILE two-dimensional electrophoresis is unsurpassed by any other technique for simultaneously resolving hundreds of polypeptides, its potential to contribute to our understanding of molecular processes remains largely untapped. Because of the difficulty in achieving adequate reproducibility and truly high resolution, the technique has successfully been practised in a relatively small number of laboratories. Those difficulties notwithstanding, it was evident at a two-dimensional electrophoresis meeting held in Vienna last November* that the technique has been used to identify a good number of novel proteins that play a role in cell activation, proliferation, differentiation and other functions[1]. Genes corresponding to proteins detected on two-dimensional gels have been cloned by several groups, including our own.

Immobilized pH gradients

A major contributor to the variability seen in two-dimensional gel patterns within and between laboratories is the carrier ampholyte (CA) which is the determinant of the first dimension separation, based on charge. Detailed comparisons of patterns generated for the same cell material in separate laboratories have been difficult, hampering efforts to establish collective protein databases of two-dimensional gel information. Problems with CA-based separations include: gaps in the gel patterns, resulting from discontinuities in the pH gradient, which varies between sources of CA; drift in the focusing pattern during electrophoresis (particularly severe for basic polypeptides); and difficulty in producing pre-cast gels suitable for two-dimensional electrophoresis.

These difficulties prompted us to develop a two-dimensional approach that utilizes immobilized pH gradients (IPG) for the first dimension separation[2,3]. With IPG, the pH gradient is an integral part of the polyacrylamide gel matrix[4]. The technique overcomes many of the problems encountered when using carrier ampholytes. The pH separation range can be defined from broad to extremely narrow, basic polypeptides can be resolved well, and precast gels can be used. IPG gels can also be loaded with preparative amounts of protein suitable for polypeptide sequencing, because they have higher loading capacities than CA gels.

In the IPG method, gradient slab gels of 0.5 mm in thickness are first cast on a GelBond PAG film in a large format (24 cm wide with a 17-cm pH gradient separation distance). For most applications, the gradient spans three pH units: 4–7 for acidic and neutral polypeptides, and 6.5–9.5 for neutral and basic molecules. Each slab gel yields roughly seventy 3.5-mm-wide first dimension strips. Following extensive washing with deionized water, the gels are dried and wrapped in thin plastic film, and can be stored at −20 °C for several months.

Prior to the second dimension electrophoresis step, the desired number of strips are cut and rehydrated, as a group, with a solution of urea, non-ionic detergent, dithioerythritol and dilute acetic acid. The strips are then placed on the cooling plate of a humidified, sealed electrofocusing apparatus, and sample applicators of between 40 and 80 µl in capacity are placed directly onto the surface of the gel.

The degree of sample entry into the gel is critical. We have found that the best results are obtained using dilute samples with a low salt concentration to which carrier ampholytes have been added, with

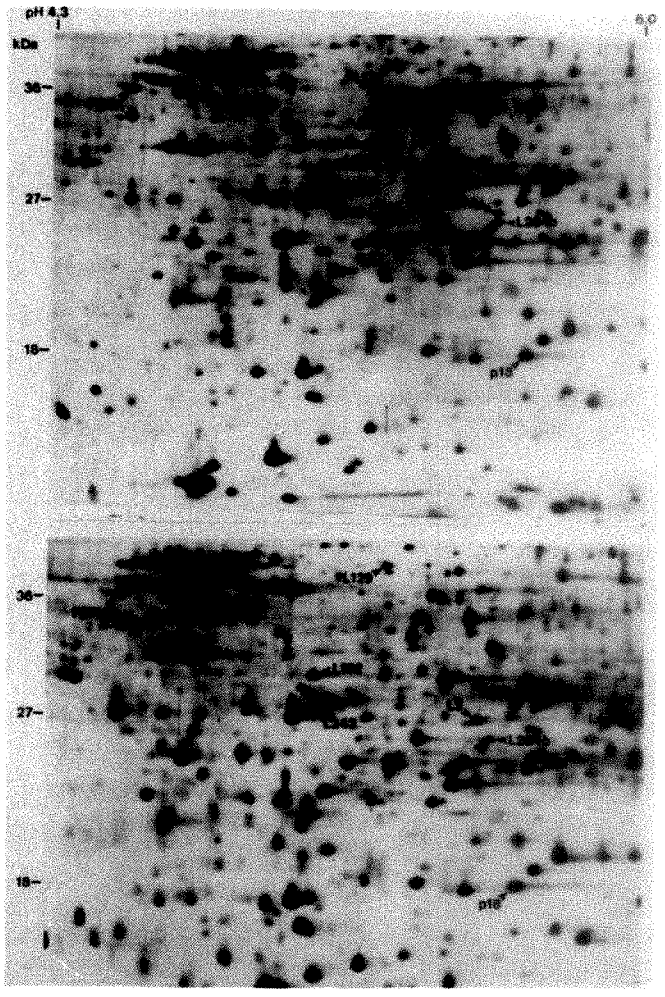


Fig. 1 Comparison of the polypeptide patterns of two separate Epstein–Barr virus-transformed lymphoblastoid cell lines, generated with two-dimensional gel electrophoresis using immobilized pH gradient gels prepared from separate castings. Arrows point to polypeptides of interest to the study of acute leukaemia, some of which have been sequenced and their corresponding genes cloned[6,7]. Some of the differences between the protein patterns are due to genetic polymorphisms, and others to stage of differentiation[7,8].

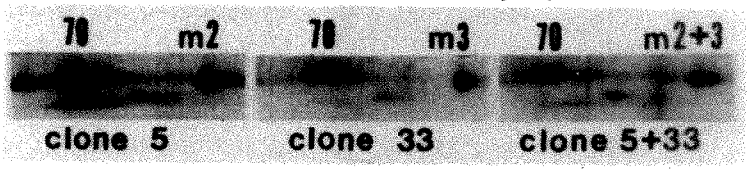


Fig. 2 Close-up sections of two-dimensional gels prepared with narrow immobilized pH gradients (pH 6.3–6.8) in the first dimension. Two mutants of polypeptide 70 prepared from separate mutagenized lymphoblastoid cell lines phenotypically identical in broad-range CA-based gels are clearly resolved by immobilized pH gradients. The mutants differ in charge by <0.003 pH units.

* The International 2-Dimensional Electrophoresis Meeting, Vienna, Austria, 8–11 November 1988.

a low voltage gradient across the gel (30 V cm^{-1}) for the first hour. The voltage can then be increased to 5,000 V (0.1 mA per strip, maximum) for 22–24 hours.

The second dimension electrophoresis step can be performed using vertical or horizontal slab gels. Figure 1 illustrates the reproducibility and resolution which can be achieved using relatively broad pH gradients. An example of the resolution which can be achieved using narrow gradients is shown in Fig. 2.

Other developments

It should be emphasized that in practice, no one technique can lead to the simultaneous visualization of all cellular polypeptides. Thus, for some applications, the selective analysis of protein subsets of interest (such as the low-abundance soluble nuclear proteins) by prior cell fractionation is advantageous.

Additional approaches include the use of separation modes other than isoelectric focusing for the first dimension separation. The direct coupling of microbore HPLC to SDS electrophoresis is particularly promising. In such a system, various HPLC separation modes, from reversed phase to ion exchange, could be used to perform the first dimension separation. In a previous study of plasma proteins, we observed that the resolving power of ion exchange HPLC is equivalent to isoelectric focusing with CA, based on the separation of transferrin variants[5].

Another promising development is the use of capillary electrophoresis as the first dimension separation mode. An effort is also being made to overcome some of the problems experienced with CA-based separations to eliminate gaps in the gels and to allow the production of precast focusing gels containing urea which can be stored for lengths of time.

It is becoming clear that, after an initial lag behind DNA technology, most of the practical knowledge is now in place to allow a major leap forward in our understanding of the organization, structure and function of the principal cellular components which mediate cell function, namely, the proteins. □

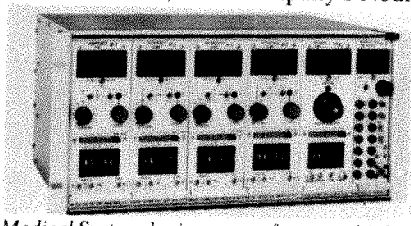
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Of peptides and DNA

Neurobiology and neurochemistry will be the talk of the town in Miami ne week during the Miami Bio/Technology *Winter Symposium. Besides an arr of patch-clamps and recorders, the exhibits will feature products for studyir peptides, and the nucleotides which code for them.*

MEDICAL Systems Corporation has a new **micropump system** for controlling the ejection of fluids from micropipettes in neurobiotechnological studies *(Reader Service No. 101)*. The company's Neuro-



Medical Systems' micropump for neurobiology.

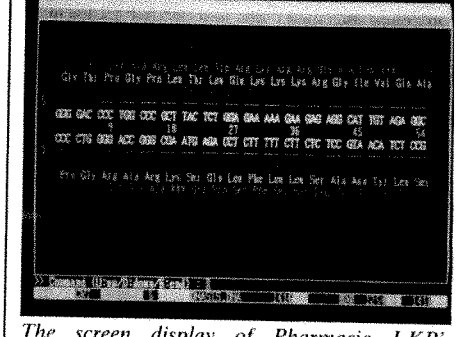
Phore BH-2 has both a pneumatic and an ionophoretic pump module, and can deliver intracellular or extracellular ejections of minute volumes using up to five pumps in parallel. The ejection schedule for each pump can be programmed independently. Medical Systems Corporation also offers multibarrel micropipettes for use with the NeuroPhore BH-2 system to enable researchers to eject several different fluid combinations for investigating cell responses. The company is exhibiting in booth 78.

United States Biochemical, exhibiting in booths 62 and 63, offers a range of **neuropeptides** which affect the central nervous system *(Reader Service No. 102)*. The company is noteworthy for producing Bombesin and MBHA-peptide resin, the preferred resin for producing alpha-carboxamide peptides, but they also sell endorphins, delta sleep-inducing peptide, alpha- melanocyte stimulating hormone, and enkephalins. Purified amino acids and derivatives for peptide synthesis can also be chosen from USB's product listing, which contains over 6,000 products. The company has recently been appointed by Perkin- Elmer Cetus as a co-distributor of Perkin-Elmer Cetus's GeneAmp reagents for performing the polymerase chain reaction.

Software smarts

Beckman has updated its popular MicroGenie **sequence analysis software** to include the capability to calculate circular restriction maps, including double and multiple digests *(Reader Service No. 103)*. The new feature allows the user to enter and store lists of restriction enzymes and their recognition sites, even before analysis, and to calculate restriction fragment sizes based on gel mobilities. The new version retains the old MicroGenie's shotgun DNA sequencing function, whe sequences can be merged and scanned f overlaps, insertions, deletions and mi matches, and the homology search ar alignment function, which draws upon th GenBank nucleic acid database and th National Biomedical Research Found tion protein sequence database. Wit MicroGenie, it is also possible to predi protein secondary structures and prob bilities, determine residue and codo frequency, predict and plot protein hydr phobicity, and find inverted repe regions and calculate the free energ Beckman offers a free demonstration dis for MicroGenie, which runs on IBM compatible computers. The company i exhibiting in booths 29 and 30.

Pharmacia LKB, exhibiting in booth 88 and 89, has just launched its PROSI **protein analysis software** package fo personal computers *(Reader Service No 104)*. The software can perform secondar structure predictions, based upon th Chou-Fasman and Robson theories; cal culate the maximum amino acid homolog



The screen display of Pharmacia LKB's DNASIS.

between two sequences; perform molecular weight and homology searches; plot homologies; and perform hydrophobicity analyses. PROSIS has keyword search and database access functions, and can be used with an optional CD-ROM disk that contains both the GenBank and National Biomedical Research Foundation databases. Version 3.0 of Pharmacia-LKB's DNASIS DNA analysis software has also recently been released. DNASIS has been updated to perform homology searches in one-third of the time required by previous versions, and to incorporate improved colour graphics. The new version also allows the automatic entry of fragment migration distances from an optional sonic digitizer for the creation of restriction

maps in either circular or linear formats.

IntelliGenetics last year released version 3.0 of its KeyBank data bank of **protein and nucleic acid sequence patterns** for use with the company's suite of software for mainframe computers *(Reader Service No. 105)*. KeyBank consists of over 1,200 functional and structural sequence patterns — referred to as "keys" — derived from protein and nucleic acid sequences published in the scientific literature. IntelliGenetics says KeyBank gives researchers a more efficient way to examine the burgeoning nucleic acid and protein sequence databases. KeyBank contains 16 categories of protein keys, including amino acid modifications, signal sites, binding sites, and enzyme and allosteric sites, and 112 categories of nucleic acid keys, including DNA structure, regulatory regions, binding sites, repeats and ends. IntelliGenetics will have information on KeyBank in booth 27.

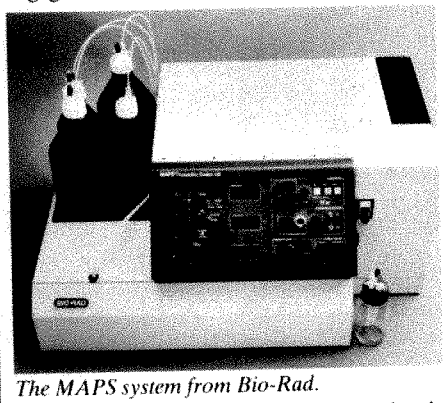
DelPhi is the name of a soon-to-be-released software package from Biosym for calculating the **electrostatic potential** of macromolecules *(Reader Service No. 106)*. *DelPhi* enables users to examine the effects of charge distribution, ionic strength, and dielectric constant on the electrostatic potential of a macromolecule without resorting to dielectric models or including explicit solvent. There is no limit on the number of atoms, residues or molecules that can be treated. Biosym says the software yields results that agree within five per cent with analytic solutions for simple spherical models. The output electrostatic potential grids may be viewed as 3-dimensional contour maps using Biosym's *Insight* modelling software. *DelPhi* also generates a dielectric map identifying points inside and outside a molecule's solvent- accessible surface, and a list of potentials at selected coordinates and total electrostatic energy. Biosym is in booth 87.

Pure protein (and DNA)

J.T. Baker, in booth 69, has a new **non-porous polymeric matrix** named Natrix for the purification and concentration of DNA and RNA *(Reader Service No. 107)*. Separation with Natrix is achieved by the selective binding of the nucleic acid to the phase in an ion-pairing buffer. The minimum capacity of the matrix is 50 μg of nucleic acid for 200 mg of Natrix. The nucleic acids are eluted using 60 per cent acetonitrile. Baker says Natrix allows the recovery of normal nucleic acids in quantitative amounts, without impairing their capacity for ligation. According to the company, Natrix can be used to purify ten samples of DNA from a caesium chloride gradient in less than 30 minutes. Natrix is available in prepacked Bakerbond columns or as a bulk sorbent.

Boehringer Mannheim Biochemicals, in booths 81-83, has introduced Resorufin-labelled casein, a non-specific substrate for detecting a wide variety of **proteases** that contaminate various protein preparations *(Reader Service No. 108)*. The company says the new substrate is both reliable and sensitive in the determination of protease activity: each lot is tested with sequencing-grade trypsin, and as little as 0.1 μg trypsin can produce an absorbance difference of 0.07 at 574 nm in 15 minutes at 37 °C.

Bio-Rad recommends its MAPS preparative system for the **process-scale purification of protein** *(Reader Service No. 109)*. Bio-Rad says the MAPS can be used in its integral sanitation method for purifying grams of monoclonal antibodies and



The MAPS system from Bio-Rad.

other proteins per day in a completely sanitized environment. The MAPS system can be treated with 1 M sodium hydroxide, and has an IBM-compatible computer for system control, data collection and manipulation, methods development, and automated sample injection. Compatible column chemistries include Bio-Rad's Affi-Prep protein A columns for MAb's, HRLC MA7 ion-exchange columns for high-molecular weight proteins, macroporous ABCM cation exchange columns for concentration from tissue culture media, and Affi-Prep polymyxin supports for removal of endotoxins. Bio-Rad is exhibiting in booth 3.

Cloning and sequencing

Promega, in booth 54, offers its TaqTrack sequencing system — based on the thermostable DNA polymerase from *Thermus aquaticus* — for sequencing applications *(Reader Service No. 110)*. Promega says as a sequencing enzyme, *Taq* exhibits consistent band intensity, low background, and a high degree of accuracy. Because *Taq* works at 70-95 °C, the TaqTrack system decreases the secondary structure of DNA templates, permitting polymerization through highly structured regions. The system is optimized to produce readable sequence data from 1 to 500 bases with a single-stranded DNA template. A deaza nucleotide mix which substitutes 7-deaza dGTP for GTP is available for resolving band compressions associated with GC-rich templates.

BRL has two **cDNA cloning systems** for cloning double- stranded cDNA into the bacteriophage vectors λgt10 and λgt11 in as little as three days *(Reader Service No. 111)*. Based on *Eco*R1 linker addition, both cloning systems guide the researcher through all of the cDNA cloning steps: methylating internal *Eco*R1 sites, repairing DNA termini, phosphorylating linkers, ligating linkers to DNA, digesting linkers, removing linkers by chromatography, and ligating DNA to the vector. Each step is outlined in the manual, which also includes troubleshooting instructions. The systems include positive control DNA to monitor the success of each step which makes phage with insert DNA form blue plaques and phage containing linkers remain colourless. Each system contains reagents for performing five reactions, with 0.5–2.5 μg cDNA per reaction. BRL is in booths 76 and 77.

The Base Runner **multi-gel electrophoresis sequencer** from International Biotechnologies, Inc. accommodates the independent running of two 32-lane gels simultaneously *(Reader Service No. 112)*. The Base Runner holds two 20.3 cm-wide gels of either 30, 45 or 60 cm in length. It has a lockable base which can be rotated 360°, and a buffer tank clamping system that IBI says eliminates user error and plate breakage while reducing setup time. The spring-loaded clamp allows users to slide tanks into place: releasing the clamp causes the tank's silicon gasket to compress against the glass plate, forming a leakproof seal. The Base Runner's gel sandwich is composed of a specially treated notched glass plate and a glass plate coated with a thermoconductive material that IBI says prevents "smiling". IBI is exhibiting in booth 43. □

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Turning the switch on skills

Richard Pearson

Industrial concerns may have to rethink their in-house training programmes — or pay dearly for the skilled workers they need.

WITH the dramatic downturn in the numbers of school-leavers available to enter the labour market and higher education systems of most Western countries, (*Nature* **355**, 100; 1988), attention is turning to the existing workforce: after all, 70 per cent of those who will be in employment at the end of the century are already in the workforce.

In response to the growing shortage of technical staff, a number of companies are now embarking on special initiatives to retrain non-technical staff and are widening their recruitment net to embrace new types of trainees by setting up conversion courses. Others are introducing special career streams for technical staff to improve their long-term career prospects, and hence retention rates (*Switching on Skills*, NEDD, 1989).

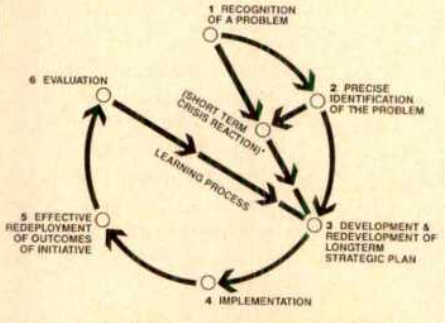
An example of one of these initiatives can be found at the BBC (British Broadcasting Corporation) which has a continuing need to recruit engineering staff to maintain its broadcasting and transmission activities. In the early 1980s the corporation started experiencing increasing difficulty, along with most other employers, in attracting sufficient engineering graduates and school-leavers qualified in mathematics and physics to enter its training schemes. It foresaw these difficulties worsening, and so decided to widen and adapt its graduate training scheme to include arts graduates. When the places were advertised there was a massive response with 8,000 enquiries, and 3,000 applications for the 24 places. An 'O'-level in maths at grade B, or better, was deemed to be the minimum technical knowledge needed and special training tests were used to aid the selection process.

In this way candidates were assessed in near normal training conditions to evaluate their suitability and probable success in the longer term. A special programme was developed to give the trainees the basics of physics in a three-week module. They then followed the 'A'-level trainee programme, both streams eventually merging with the engineering graduate group. The initial off-the-job part of the programme lasts about 13 weeks, and is followed by an 18-month period embracing both off- and on-the-job training.

Although still in its early stages, the programme has been deemed a success, with none of the trainees failing. The BBC has found a completely new source of recruits, reducing their need to compete in the market for experienced engineers. It is argued that as overall salary levels do not have to match the top of the market, the training costs involved, which exceed £10,000 per trainee for the initial 13 weeks alone, are justified.

Another approach to widening the recruitment net and using non-technical recruits has been undertaken by part of the electronics group Plessey. A shortage of real-time programmers was the spur here, and the company looked to retain internal staff. An in-house 26-week course was designed which could not only take students from a wider range of backgrounds than the external equivalent but was also 60 per cent cheaper. It includes foundation elements of maths and electronics as well as the basics of software. This is followed by short modules on specialist high-level languages and project work. Staff and ex-students have described it as compressing two years of university study into six months.

Initially the course was aimed at graduates but more recently a base entry standard of good 'O'-levels was accepted



The best of training programmes could fail if it is not adapted in the light of experience.

for staff who might otherwise have been in danger of redundancy elsewhere in the company. The course was externally validated and in the second year the Manpower Services Commission, a government agency, arranged to buy places to retain graduates and to help ease the more general skill shortages. Selection was by aptitude test and interview with a notional target age range of 20–30 years, reflecting the perceived market prejudice against older workers. All 90 of the students taking part so far have successfully completed the course; the benefits of 'growing your own' having been emphasized by high retention rates. For the externally sponsored students, normally graduates with no information technology experience and from backgrounds ranging from civil engineering to the arts having been in employment for up to 5 years, the course were seen to be highly practical. Their relatively short duration, when compared with a 12-month MSc, means that students can find financial support more easily. By allowing externally sponsored students to participate, the company has been able to continue with the course even in times of slack internal demand.

At Barr and Stroud, manufacturers of electro-optical systems, the concern was to provide career routes for specialist staff and improve retention by allowing career progression outside the traditional management hierarchy. The company has introduced a number of new programmes including a career structure with three streams for professional, specialist and managerial staff, explicit grade and progression criteria, an integrated and visible salary structure, and an appraisal system. The latter is a critical means of identifying training needs and succession planning. The programme, which is currently being implemented, will, it is hoped, result in clearer and better career prospects for staff, leading to lower staff turnover.

For other companies interested in developing such initiatives there are a number of lessons of value from these and the complementary case studies (presented in *Switching on Skills*). The first is the need is to recognize the nature and durability of the problem, such as recruitment difficulties, high wastage or poor utilization. Often this is done but only leads to short term, or crisis reactions. The real need is to identify the precise problem and develop a strategic response. Implementation of the plan then follows; it is at this stage that many initiatives stop. In many cases, however, staff are retrained and then their new skills are not exploited. The programme needs to be evaluated and the lessons fed back into the design and implementation strategies (see figure). Few programmes are 100 per cent successful the first time, and even where they are the problem or the environment will continue to change. The challenge now is to increase the skills of the existing workforce to complement the new skills provided by the young. □

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植物工学・医薬開発分野の経験ある研究者

サッポロビール㈱は「ビールを基軸とした複合経営」を目指し、都市再開発から医薬および農業関係に至る広い分野でR&Dに取り組んでいます。このたび、展開を加速するために全社的な組織改革を行いました。これにともない右記の研究所では戦力増強を計画しています。

募集要項

■植物工学研究所(群馬県新田郡)
●植物の遺伝子工学研究者(チーム・リーダー)……1名
上記分野の研究歴5年以上。年齢40歳位。博士号必要。
微生物遺伝子工学経験者で植物に関心のある方も可。

■医薬開発研究所(静岡県焼津市)
●医薬品合成、薬理、微生物スクリーニングの各主任研究員クラス……各若干名
経験3年以上。年齢30歳前後。

《応募方法》
履歴書(写真貼付)、研究歴要旨および論文別刷を下記にご送付ください。

☞郵送・問合先:〒104 東京都中央区銀座7-10-1 サッポロビール株式会社
研究開発本部(近野/高塩)☎(03)572-6111 Fax(03)572-0221

JAPAN'S OLDEST BRAND SINCE 1876
SAPPORO BEER

(W5807)A

HIGHER SCIENTIFIC OFFICER (BAND I)
(21-month appointment)
TISSUE CULTURE AND CYTOLOGY DEPARTMENT

A plant scientist is required to explore the potential of asymmetric cell fusion in potato improvement. Knowledge of plant tissue culture techniques is required and experience of protoplast manipulation would be an advantage. A technical support worker is also available for this position.

Salary: £10,026–£13,460

Non-contributory superannuation.

Qualifications: First or upper second class honours degree in a related discipline with at least two years' relevant post-graduate research experience which may be a course of research and study leading to a Ph.D. degree.

The Institute is an equal opportunities employer.

Curriculum vitae complete with the names and addresses of three refereees should be sent to **Mr I Paxton, Personnel Officer, by 24 February 1989** quoting the appropriate reference (**Ref. TCC/1/89**).

(8279)A

Universität zu Köln

An der Mathematisch-Naturwissenschaftlichen Fakultät der Universität zu Köln ist zum Sommersemester 1990 am Institut für Entwicklungsphysiologie eine

Professur C3
fur Entwicklungsbiologie

wieder zu besetzen.

Bewerber (innen) sollen sich durch ein aktives Forschungsprogramm auf dem Gebiet der molekularen Grundlagen der Entwicklung des Nervensystems (vorzugsweise von Insekten) qualifiziert haben. Eine Mitarbeit im Sonderforschungsbereich 243 "Molekulare Analyse der Entwicklung zellulärer Systeme" ist möglich. Eine Beteiligung an den Lehrverpflichtungen des Instituts wird erwartet.

Bewerbungen mit Lebenslauf, Schriftenverzeichnis, einschließlich Sonderdrucken der wichtigsten Publikationen und kurzem Forschungsplan werden bis zum 15. April 1989 erbeten an den **Dekan der Mathematisch-Naturwissenschaftlichen Fakultät der Universität zu Köln, Albertus-Magnus-Platz, 5000 Köln 41.**

(W5812)A

BROWN UNIVERSITY

Providence, Rhode Island ● 02912

Division of Biology and Medicine

BROWN UNIVERSITY

PROFESSOR AND CHAIRPERSON, SECTION OF PHARMACOLOGY

The Division of Biology and Medicine plans to appoint a Professor and Chairperson of Pharmacology during the academic year 1989-90. With the construction of a new wing to the Biomedical Center, opportunities exist to develop the current program in biochemical pharmacology into a broadly-based, unit in pharmacological sciences, which interacts with programs in biochemistry, molecular biology, neurobiology, pathology, and clinical pharmacology. The successful applicant is expected to lead this development, to establish a vigorous research program of national distinction, and to take administrative responsibility for pharmacology teaching at Brown. Pharmacology faculty participate in medical, graduate, and undergraduate teaching. The integrated structure of the Division of Biology and Medicine maximizes opportunities for research and teaching interaction among all of the biological and medical sciences, as well as with other disciplines at Brown University. Applicants should submit a curriculum vitae, representative reprints, and the names of three references to **Dean Frank G. Rothman, Division of Biology and Medicine, Brown University, Providence, RI 02912**. The deadline for receipt of these application materials is March 31, 1989. *An Equal Opportunity/Affirmative Action Employer.* (NW3263)A

GRIFFITH UNIVERSITY

Division of Science and Technology

LECTURER

Pharmacology/Physiology
(Continuing Appointment)
Ref. No. F14-88

Applications are invited for a lectureship in the areas of Pharmacology/Physiology in the Division of Science and Technology. The successful applicant will be expected to teach pharmacology at the undergraduate and postgraduate levels and to contribute to physiology teaching at undergraduate levels.

Applicants should possess a suitable higher degree and a record of research in an appropriate area. Some teaching experience would be an advantage.

The appointee will be expected to commence duties from 1 November 1989. It is anticipated that the appointment will be made in the lower half of the lecturer's salary range, i.e. A$30,737-$34,750.

Applications from women and men are encouraged. Employment benefits include parental leave and the possibility of access to full-time child care and after-school care.

Further details may be obtained from the Divisional Administrator, Division of Science and Technology, to whom applications (including curriculum vitae and names and addresses of two referees) should be sent by 31 March 1989.

Griffith University Nathan Qld 4111 AUSTRALIA

AN EQUAL OPPORTUNITY EMPLOYER (W5825)A

THE UNIVERSITY OF CALIFORNIA IRVINE CANCER CENTER

is seeking an assistant adjunct professor of medicine. Cancer research with expertise in molecular and cell biology. Study of transformation of human melanocytes and chemoprevention. Funding for at least 2 years available. Opportunity to develop independent career. Postion available July 1, 1989.

Send letter, list of at least 3 references and curriculum vitae to: **Faculty Recruitment (Laboratory) Frank L. Meyskens, Jr., M.D., Director, UCI Cancer Center, 101 The City Drive, Building 44, Route 81, Orange, CA 92668.** Applications accepted until April 1, 1989 or until position is filled.

UCI is an EOP/AA employer. (NW3190)A

UNIVERSITY OF OXFORD

GLYCOBIOLOGY UNIT

POSTGRADUATE RESEARCH ASSISTANT 1B

BR/283

Applications are invited from suitably qualified graduates for the post of Research Assistant 1B to join an active group studying the structure and function of glycoproteins. The successful candidate will be involved in the characterization of oligosaccharides from diverse sources and in associated support duties. Previous experience is not essential, but an understanding of various chromatographic, radiolabelling and computing techniques would be an advantage.

The appointment offers promising career opportunities in this exciting field, and is available for 3 years in the first instance.

Salary on the scale £8,675-£13,365 p.a.

Applications, including a curriculum vitae and the names and addresses of 2 referees, should be sent to: The Administrator

An Equal Opportunity Employer. (8286)A

The University is an Equal Opportunity Employer

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY

LECTURER IN PHYSICAL CHEMISTRY
LECTURER/SENIOR LECTURER/ READER IN POLYMER CHEMISTRY

Applications are invited for two permanent posts from October 1989.

(a) Lecturer in physical chemistry. The successful candidate should have research interests which suitably complement or supplement the strengths of the Department, some of which are (i) solid-state and surface chemistry, (ii) spectroscopy, (iii) molecular interactions, (iv) kinetics and mechanism. Preference may be given to applicants with research interests in polymer materials.

(b) Lecturer/Senior Lecturer/Reader in polymer chemistry. The post is open to candidates with organic, inorganic or physical chemistry backgrounds, but an established research reputation in polymer chemistry is essential.

The initial salaries will be on the appropriate scales according to qualifications and experience, Lecturer A (£9,260–£14,500) or Lecturer B (£15,105–£19,310), Senior Lecturer/Reader (£20,270–£22,910).

Further particulars may be obtained from the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE (Telephone (091) 374 2263)** to whom applications (three copies), including the names of three referees, should be sent not later than *Friday, 17th March, 1989* (quoting ref. C1). (8294)A

POSTDOCTORAL FELLOW

Postdoctoral positions are available in a molecular biology-electrophysiology laboratory for research on the structure-function relationship of brain and cardiac sodium, potassium and calcium channels. Site-directed mutagenesis and hybrid chimeras are used to construct channel mutants for expression in Xenopus oocytes and stably transfected cell lines are being established. Channel technology would be helpful. Interested applicants should send resume and addresses for three letters of reference to:

Rolf H. Joho, Ph.D., Assistant Professor or Arthur M. Brown, M.D., Ph.D., Professor and Chairman, Department of physiology and Molecular Biophysics, Baylor College of Medicine, One Baylor Plaza, Texas Medical Center, Houston, Texas 77030.

We are an equal opportunity employer. (NW3213)A

PUBLIC HEALTH SERVICE
ALCOHOL, DRUG ABUSE AND MENTAL HEALTH ADMINISTRATION
NATIONAL INSTITUTE OF MENTAL HEALTH
NEUROPSYCHOPHARMACOLOGIST

The Neuropsychiatry Branch of the NIMH, under the leadership of Dr. Richard Wyatt, is recruiting for a neuropsychopharmacologist. The individual selected will participate in ongoing basic science studies regarding the CNS effects of a variety of psychoactive drugs, with an initial focus on cocaine, caffeine, and nicotine. These studies will include examinations of the impact of these drugs on neurotransmitter-receptor systems and the design of animal models to assess behavioral effects. Applicants must have a doctoral degree in pharmacology or a related area, and preferably have specific experience in behavioral pharmacology.

Applicants may be accepted as Staff Fellows ($20,000 - 38,000) or Visiting Fellows ($20,000 - $23,000). Staff Fellows must be U.S. citizens with a doctoral degree. Initial appointments are for two years, with further renewals possible. Visiting Fellows may be resident or non-resident aliens with a doctoral degree and no more than three years of relevant postgraduate experience. Visiting Fellows are appointed for one year with possible renewals for two additional years. Interested candidates should send a curriculum vitae, a brief statement regarding experience and research interests, and the names of 3 references to:

Darrell G. Kirch, M.D.
NIMH Neuroscience
Center at Saint Elizabeths
2700 Martin Luther King Jr.
Avenue, S.E.
Washington, D.C. 20032
(202) 373-6097

NIMH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW3249)

NATIONAL JEWISH CENTER FOR IMMUNOLOGY AND RESPIRATORY MEDICINE

EICOSANOIDS AND PAF RESEARCH

University of Colorado Health Sciences Center

Postcoctoral positions in eicosanoids and platelet activating factor research are currently open in a joint program in the laboratories of the National Jewish Center for Immunology and Respiratory Medicine and University of Colorado Health Science Center in Denver, Colorado. Individuals interested in any of the following projects are encouraged to apply.

'Suicide' Inactivation of Leukotriene A Hydrolase: The objective is to characterize the autoinactivation process that limits formation of the chemotactic agent leukotrianes B_4. The results will facilitate efforts to monitor leukotriene biosynthesis *in vivo* and to comprehend transcellular metabolism of leukotrienes. [See Proc. Natl. Acad. Sci. USA *83*;1349 (1986)]. Contact F.A. Fitzpatrick, 303-270-7494, UCHSC.

Leukotrianes and Platelet Activating Factor: An individual interested in learning and then applying sophisticated techniques of lipid biochemistry, including mass spectrometry, will study the source of arachidonate and involvement of ether phospholipids in eicosanoid and PAF biosynthesis. Experiments will be carried out with inflammatory cells such as mast cells and neutrophils using physiologically relevant stimuli to trigger lipid mediator biosynthesis. [See J. Biol. Chem. *261*;7771 (1986)]. Contact Robert C. Murphy, 303-270-8962, UCHSC.

Platelet Activating Factor in Acute Lung Injury: An individual interested in the mechanisms of lung vascular injury will study hemodynamic effects of PAF and its synergistic interactions with proteases and oxidants and injurious effects of PAF on cultured vascular cells. [See J. Clin. Invest. *79*:1498 (1987)]. Contact N. F. Voelkel, 303-270-8103, UCHSC.

Phospholinase A_2 Involved in Eicosanoid and Platelet Activating Factor Production: The project involves studying the biochemical properties and mechanisms of activation of an arachidonoyl-hydrolyzing phosphalipase A_2 from macrophages. [See Biochem. Biophys. Acts *963*;476 (1988)]. Contact Christina C. Leslie, 303-398-1214, NJCIRM.

Secretion of Lipid Mediators From Cells: The mechanism by which newly synthesized eicosanoids, precursors and PAF are liberated from cells is the objective of these studies. Techniques of cell biology, biochemistry, mass spectrometry, and membrane biophysics will be employed to answer these questions. Contact Peter Henson, 303-398-1380, NJCIRM.

Send or FAX (303-270-7097) letter of interest to the above Professors (UCHSC) or to Prof. P. M. Henson (NJCIRM).

National Jewish Center for Immunology and Respiratory Medicine
1400 Jackson Street
Denver, Colorado 80206

University of Colorado HSC
Department of Pharmacology (C236)
4200 E. 9th Avenue
Denver, Colorado 80262

EOE/AA

(NW3252)A

SCRIPPS CLINIC
AND RESEARCH FOUNDATION

RESEARCH INSTITUTE OF SCRIPPS CLINIC
LA JOLLA, CALIFORNIA, U.S.A.

MOLECULAR BIOLOGIST
MEMBRANE BIOCHEMISTRY

A staff position is immediately available for an individual trained in the field of molecular biology with experience in protein chemistry. Studies are already in progress on the molecular mechanisms in leukocyte membranes responsible for transduction of signals initiated by ligand-receptor-G protein interactions. Precise understanding of these events, and experimental manipulation of the system requires the participation of molecular biologists. Considerable scholarly and educational resources are associated with the position. Excellent salary, fringe benefits, grant and technical support are available. Send curriculum vitae, description of research background and research interests, education and training to:

Charles G. Cochrane, M.D.
Department of Immunology, IMM12
Research Institute of Scripps Clinic
10666 North Torrey Pines Road
La Jolla, CA 92037 U.S.A.

Equal Opportunity Employer.

(NW3253)A

FACULTY POSITIONS
SLOAN-KETTERING INSTITUTE
CELL BIOLOGY AND GENETICS PROGRAM

The Sloan-Kettering Institute invites applications for several tenure-track junior and senior faculty positions in the Cell Biology and Genetics Program. This research program is one of four in the Institute, and is undergoing a major expansion in research at the molecular level on the areas of positive and negative growth control, differentiation, cell structure, intercellular recognition, and morphogenic pattern formation. The Cell Biology Program and the Molecular Biology Program at the Sloan-Kettering Institute are jointly relocating to a new research building which also houses advanced facilities for protein and nucleic acid microchemistry, transgenic mice, and automated mammalian cell micromanipulation. The faculty members of the Cell Biology and Genetics Program are also affiliated as faculty with the Cell Biology Graduate Program at Cornell University Medical School. The positions offered are highly competitive with regard to start-up funds, laboratory space, salary and housing.

Eligible candidates should have a doctoral degree, post-doctoral experience and demonstrate potential and ability to develop an independent, imaginative research program. Formal applications are invited from junior as well as established investigators. Applications should include a curriculum vitae, a list of publications, a summary of research activities and research plan, and the names of three or more references. Applications should be addressed to: **Dr. Joan Massague, Cell Biology and Genetics Program, Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021.**

(NW3199)A

KING'S COLLEGE LONDON

Biomolecular Sciences Division

POST DOCTORAL POSITION IN PROTEIN CRYSTALLOGRAPHY

Applications are invited for a three year post-doctoral position funded by the MRC, for work on the three dimensional structure of a metallo-β-lactamase enzyme, and its interaction with penicillin and cephalosporin substrates at low temperature. The applicant will also be involved in the structure determination of a penicillin target protein, and its complexes with β-lactam antibiotics.

Salary £11,515-£12,720 including London allowance, according to age and experience. Applications, together with a c.v. and the names of two referees should be sent to **Mr D.M. Drummie, 26-29 Drury Lane, London WC2B 5RL**. Informal inquiries may be directed to Dr B.J. Sutton (01-836 8851), from whom further details may be obtained.

(8284)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA

U.G. 7

HEAD OF CEREAL AND LEGUME GENETICS DEPARTMENT

Applications are invited for the post of Head of the Cereal and Legume Genetics Department of SCRI. The postholder will manage a programme aimed at elucidating the genetic architecture of characters of economic and biological importance in barley and Vicia beans, and at developing genetic improvement strategies. Particular attention is given to the components of yield, disease resistance and malting quality (in barley) and anti-metabolites (in beans). Research includes the use of molecular and biometrical genetics, tissue culture and modelling of physiological and morphological development.

The Department consists of seventeen staff (1 UG7, SSO, 2 HSO, 2 SO, 5 ASO, 5 EW) and is located at Mylnefield, Dundee where excellent laboratory, glasshouse and field facilities are available.

Salary: £16,692–£21,737

Non-contributory superannuation.

Qualifications: Preference will be given to candidates with post-graduate qualifications in genetics and an interest in utilising the most recent technologies of gene manipulation.

The Institute is an equal opportunities employer.

Curriculum vitaes complete with the names and addresses of three referees should be sent to Mr I Paxton, Personnel Officer, by 17 February 1989 quoting the appropriate reference. (Ref. C&LG/1/89) (8281)A

University of Birmingham
School of Basic Medical Sciences

Lecturer in Anatomy

Applications are invited for the above post in the Department of Anatomy which provides the teaching in topographical anatomy, neuroanatomy, histology and cell biology to medical, dental and science students. The successful candidate would be expected to contribute to teaching in one of these areas although particular skills in neurosciences will be welcome.

We are seeking a candidate who can offer evidence of a high level of research achievement and potential probably in cell biology or neuroscience, although candidates with expertise in other areas of interest within the School are encouraged to apply. Expertise in molecular biology, tissue culture, neurochemistry and electrophysiology is available in the School and ample opportunity exists for collaboration.

Salary in the range: £9,260–£19,310 p.a. plus superannuation.

Informal enquiries can be made to **Professor J.J.T. Owen, Department of Anatomy, The Medical School, University of Birmingham, Birmingham B15 2TJ (Phone: 021-414-6812) or to the Head of the School of Basic Medical Sciences, Professor J.H. Coote (Phone: 021-414-6915/6).**

Further particulars and application forms are available from: **The Senior Assistant Registrar, The Medical School, University of Birmingham, Birmingham B15 2TJ (Phone: 021-414-4049)** to whom applications, including c.v. and the names of three referees, should be sent by not later than 24th February 1989.

AN EQUAL OPPORTUNITIES EMPLOYER (8272)A

Position announcement

International distribution

GEOMAR, Research Center for Marine Geosciences which was established in 1987 at the **Christian-Albrechts-Universität in Kiel/ Germany**, invites applications for a BAT/IIa-appointment in

Marine Environmental Geology

BAT/IIa is the classification in the German civil service system equivalent to Research Associate, Senior Researcher or Research Specialist.

The successful candidate will be expected to develop aspects of a research program on the role of fluids in global tectonic and environmental settings as well as participate in the build-up of the Research Center. Desired expertise in one or more of the specialities; chemistry/hydrology of marine pore fluids, trace gases, light isotopes, sensors for dissolved tracers. Applications should be accompanied by an outline of own research interests.

Ph.D. or equivalent degree is required. Women are encouraged to apply.

Please send application, including resumé and addresses of three references, before 1 April 1989 to:

GEOMAR Research Center for Marine Geosciences
Personnel Department
Wischhofstrasse 1-3
D-2300 Kiel 14
Fed. Rep. Germany (W5811)A

POSTDOCTORAL POSITION

A postdoctoral position is available for hormonal and biochemical investigations on male sterility in plants. Candidates should have a Ph.D. in Botany/Plant Sciences with a strong background in plant development and biochemistry. Experience in plant tissue culture, protein and/or hormonal analysis would be desirable. The position is initially for one year with a possibility of renewal for the second year.

Interested candidates should send a CV, reprints/ preprints of papers and at least two letters of recommendation to: **Dr. V. K. Sawhney, Department of Biology, University of Saskatchewan, Saskatoon, SK S7N OWO, Canada.** (NW3261)A

PLANT BREEDING INTERNATIONAL

NEW OPPORTUNITIES WITHIN OUR EXPANDING SCIENCE GROUP

ASSISTANT MANAGERS/RESEARCH ASSISTANT

Two assistant managers and one research assistant are required for two different teams, one working on the molecular biology of plant viruses, and the other on the molecular biology of potato nematode disease resistance. The assistant managers will have line management responsibilities.

Qualifications: Either a HND, BSc or equivalent, and laboratory experience in molecular biology and/or biochemistry, extensive in the case of assistant managers, together with a high degree of care and reliability in experimental work.

For further particulars telephone Cambridge 840411 ext 307, or write to the **Personnel Officer, PBI Cambridge Ltd, Maris Lane, Trumpington, Cambridge CB2 2LQ quoting Ref: SCI/42/43.**

PBI CAMBRIDGE
PLANT BREEDING INTERNATIONAL

An Equal Opportunities Employer (8299)A

Pharmacia

Pharmacia Genetic Engineering Inc.— Phage —

GENE EXPRESSION — MOLECULAR BIOLOGY

Inflammation/Virology/Autoimmunity

Pharmacia Genetic Engineering Inc. — Phage — went into operation less than 2 years ago and is already poised to play a key role in Pharmacia's quest to offer to its customers, a new generation of tomorrow's health care products. The company comprises a fine team of highly qualified staff (current total, 26 individuals). Phage maintains laboratories at the Torrey Pines Center — in the midst of an environment which exhibits one of the strongest life science growth areas in the United States. We are now expanding our laboratories and are looking for

RESEARCH SCIENTISTS: PHD LEVEL

The qualified applicant must have

- Documented skills in applied molecualr biology (gene cloning, analysis, expression — yeast expression of particular interest), preferably through industrial experience
- Specific expertise in one or several fields, including autoimmunity/cancer/inflammation/virology
- The spirit of a team player
- The desire to become part of a group of achievers

Phage combines the excitement of a start up with the necessary back up by Pharmacia, with its full-fledged R&D, production and marketing organization.

If your interests and expertise meets with our needs, we should get together and tell you more. . . . Please send your CV, including three references for us to contact, to **Pharmacia Genetic Engineering Inc., Attn. Torsten B. Helting, P.O. Box 8827, La Jolla, CA, 92038.** *Confidentiality is guaranteed.*

— We are an equal opportunity employer — (NW3260)A

THE UNIVERSITY OF SHEFFIELD
Department of Human Metabolism and Clinical Biochemistry

POSTDOCTORAL/POSTGRADUATE RESEARCH ASSISTANT

Applicants are invited to apply for this post to join a group studying the behaviour of human bone cells. The position is for one year in the first instance, and the starting salary will be within the IA Range for Research & Analogous Staff £9,865–£15,720 per annum according to age, qualifications and experience.

Applicants should send a full curriculum vitae and the names/addresses of two academic referees to **Dr J A Kanis, Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX** by 20 February 1989. Ref: R862/N.

Informal enquiries may be made to Dr J A Kanis (0742-739176)

An Equal Opportunity Employer (8297)A

UNIVERSITY COLLEGE & MIDDLESEX SCHOOL OF MEDICINE
Department of Pharmacology

RESEARCH TECHNICIAN

Applications are invited for a new three year appointment as a Research Technician (Grade 3: minimum salary £8386 p.a. including London Allowance) to study the receptor-effector mechanisms for substance P and the neurokinins in bladder muscle. The post is supported by Pfizer Central Research and will be under the supervision of Dr. I. Marshall with the collaboration of Mr. E.J.C. Milroy and Mr. C.R. Chapple (Department of Urology, The Middlesex Hospital). Experience or a qualification in Pharmacology or a related discipline would be an advantage. Although based in the Pharmacology Department at UCL, a component of the research could be undertaken at the Pfizer research facilities, Sandwich, Kent.

Applicants should send a curriculum vitae including the names and addresses of two referees as soon as possible to **Dr. I. Marshall, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT.** Equal Opportunities Employer. For further enquiries please telephone: 01-387 7050 ext. 3693. (8301)A

GROUP LEADER POSITION IN MOLECULAR NEUROBIOLOGY

Friedrich Miescher-Institut Basel, Switzerland

The Friedrich Miescher-Institut, a foundation devoted to basic research in cell biology, invites applications for an independent group leader position in neurobiology. Candidates are sought who have an interest in using molecular biology techniques to study the differentiation and the regeneration of the neuronal cell in vitro and in vivo. The candidate should have at least three years postdoctoral experience in molecular aspects of cellular neurobiology. The appointee's group will join two other groups working in the field of neuronal development, led by Dr. Andrew Matus (cytoskeleton) and Dr. Denis Monard (growth factors).

The Institute has strong research programs in cellular and molecular biology and offers an outstanding scientific environment with excellent research facilities. Employment conditions for the offered position are highly competitive. The envisaged starting date is mid-1989 or later. Applications, including an outline of the candidate's proposed research project, should be submitted by February 28, 1989 to **Dr. Denis Monard, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 BASEL, Switzerland.**

(W5748)A

INSTITUTE OF PLANT SCIENCE RESEARCH
Cambridge Laboratory
Cereal RFLP Mapping Group

The Laboratory is seeking to fill three positions to expand an established and well funded team in the Cereals Research Department, which is constructing detailed genetic maps of the chromosomes of bread wheat and barley using DNA probes to identify RFLP markers. As the maps emerge, genes controlling characters of agronomic significance will be included and thus become 'tagged' for selection by breeders. The three posts will be serviced by two technicians.

The positions are funded for three years by the Agricultural Genetics Company. The technology developed will be licenced to a number of international breeding companies.

Initially the work will be conducted at Trumpington in the Cambridge Laboratory but in early 1990 the group will be relocated together with other staff, to new, purpose built laboratories on the John Innes site at Norwich.

A. Job Ref CR/348
A Higher Scientific Officer with experience in molecular biology and a knowledge of plant genetics is required to undertake a research role in the development of new probes and technology for RFLP analysis and associated techniques as applied to cereal genomes.

B. Job Ref CR/349
Two Scientific Officers are required, to organise and carry out large scale RFLP screening. Some molecular biology training is desirable for both positions. One of the posts will be suitable for a graduate with an interest in the use of computers for data base development.

Salary
Scientific Officer £8,574 pa to £10,994 (£11,477 from 1.8.89)
Higher Scientific Officer £10,026 pa to £13,460 (£13,999 from 1.8.89)
Starting point depending on qualifications and experience.

Qualifications
Higher Scientific Officer. A first or Upper Second class honours degree in a relevant plant science with at least two years post qualifying experience.

Scientific Officer. An honours degree, or equivalent, in a relevant plant science.

Three referees are required for the post of Higher Scientific Officer and two for Scientific Officer.

Applications, quoting the job reference, to the **Secretary, IPSR (Cambridge Laboratory), Maris Lane, Trumpington, Cambridge CB2 2JB, England,** by 27 February 1989.

Further particulars available on request.

The Institute is an Equal Opportunities Employer. (8305)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA

U.G. 7
POTATO BREEDING DEPARTMENT

The Department breeds potato cultivars for the UK ware market and for Mediterranean regions, where it is hoped that new cultivars could create markets for Scottish seed producers, and conducts research into breeding technologies. The breeding of new cultivars may in future become less important than the development of intermediate enhanced germplasm for distribution to commercial breeders.

The appointee will lead a team of 12 and plan and direct the germplasm enhancement work from the choice of parents to the final stages of selection and in addition will design a research programme to complement the breeding work. Integration of research findings, new technologies and refined objectives into the programme will ensure it becoming the fore-runner for breeding in the UK.

Salary: £16,692–£21,737

Non-contributory superannuation.

Qualifications: First or second class Honours degree in plant science, including genetics, and several years' post-graduate experience including a Ph.D. A keen appreciation of recent developments in genetic manipulation and the ability to adapt these to practical applications is essential as are well developed scientific leadership and management qualities.

The Institute is an equal opportunities employer.

Curriculum vitae complete with the names and addresses of three referees should be sent to Mr I Paxton, Personnel Officer, by 17 February 1989, quoting the appropriate reference. (Ref.PBG/1/89) (8278)A

La Trobe University
School of Physical Sciences
RESEARCH ASSOCIATE IN PHYSICS
(Fixed Term three years)

The appointee will work in collaboration with Professor K.D. Cole on theoretical problems in solar-terrestrial physics. The person should have expertise in relevant areas of theoretical physics and be proficient in numerical computing and simulation, have a PhD and evidence of achievement by publication in refereed journals. The appointment is for three years and the person should commence duty on 1 March 1989 or as soon as possible thereafter.

Closing Date: 17 February, 1989. Ref. No: ACA0/627

Salary: $26,617-$30,360

Applications (marked confidential) including reference number, names and addresses of three referees and curriculum vitae should be forwarded to the Staff Officer, La Trobe University, Bundoora, Victoria, Australia, 3083.

Equal Opportunity is University Policy. (W5823)A

UNIVERSITY OF LEICESTER
MOLECULAR RECOGNITION OF INOSITOL PHOSPHATE SECOND MESSENGERS BY INTRACELLULAR RECEPTORS & ENZYMES
TWO POSTDOCTORAL RESEARCH ASSOCIATESHIPS

Applications are invited for two postdoctoral research associateships supported by the SERC Molecular Recognition Initiative to work in collaboration with Dr. B.V.L. Potter (Chemistry) and Professor S.R. Nahorski (Pharmacology) on aspects of the above topic. Leicester University has been recently designated a Centre for Molecular Recognition by SERC and substantial interactive biological and chemical research is already in progress on structure–activity relationships and mechanisms of action of inositol polyphosphate second messengers at receptors regulating intracellular calcium.

One position is for three years and would be suitable for a biochemist/pharmacologist who has an interest and/or record of achievement in receptor mechanisms, the actions of second messengers and enzymology. The other position is for two years and would be suitable for candidates with expertise in organic synthesis and with a significant interest in biological mechanisms. Some of the work will be carried out within the environment of a new joint Chemistry/Pharmacology laboratory at Leicester.

Salary according to age and experience on Scale IA: £9,865 to £15,720 per annum.

Applications with Curriculum Vitae and the names and addresses of two referees should be sent before the end of February to **Dr. B.V.L. Potter (Chemistry, tel: 0533 522129) or Professor S.R. Nahorski (Pharmacology, tel: 0533 522923), University of Leicester, Leicester, LE1 7RH.** (8290)A

THE UNIVERSITY OF TEXAS Southwestern Medical Center AT DALLAS
Department of Biochemistry
POSTDOCTORAL FELLOW POSITIONS BIOCHEMISTRY AND GENETICS OF PROTEIN TRANSPORT

Positions are available to examine the molecular genetics of protein and ribonuclear protein transport across organelle membranes in yeast. Studies will focus on *in vitro* transport methods in conjunction with available genetic and immunological probes to define structure function relationships of the transport apparatus within the nuclear envelope and mitochondrial membranes. Send curriculum vitae or call 214-688-2120, **Dr. Michael G. Douglas, Department of Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75235-9038.** An equal opportunity employer. (NW3221)A

UNIVERSITY OF CAPE TOWN

Dept. of Microbiology:

Lecturer/Senior Lecturer

Applications are invited from Bacteriologists for the above post, with effect from 1 July 1989 or as soon as possible thereafter.

An interest and experience in bacterial physiology and biochemistry, anaerobic bacteriology, microbial genetics and recombinant DNA technology will be a strong recommendation.

The post may be filled on a permanent basis or on a three year fixed-period basis, whereafter it may be renewed or converted to a permanent post.

Applicants should state the level of post for which they are applying and if they wish to be considered for a temporary or permanent appointment. Applicants for the Senior Lectureship should also indicate whether they would be prepared to be considered for a Lectureship.

The salary package includes medical aid, pension and housing subsidy schemes. Details concerning remuneration will be supplied to applicants.

Applications, including a full curriculum vitae, research interests and publications, present salary, and the names and addresses of three referees, should be submitted not later than 24 March 1989 to the Registrar (Attention: Appointments Office Ref. DB), University of Cape Town, Rondebosch 7700, Republic of South Africa, (telex: 5-22208; telefax: 650-2138). Further information may be obtained from the above, or from the Secretary, SA Universities Office, Chichester House, 278 High Holborn, London WCIV 7HE.

The firm policy and practice of the University of Cape Town is to oppose discrimination in any sphere of university life on grounds of gender, race, or creed. UCT has consistently protested, and continues to protest, against apartheid laws and practices and is committed to working towards a just future for our country. Information on this is available on request.

(W5818)A Bates Wells Recruitment CT ● 737R-OS

UNIVERSITY OF DURHAM
PHYSICS DEPARTMENT
EXTRAGALACTIC ASTRONOMY

Applications are invited for a SERC funded Postdoctoral research position tenable for three years starting 1st October, 1989 (or earlier if convenient) to work with Professor R. S. Ellis and colleagues on observational programmes related to the origin and evolution of normal galaxies and the constraints these place in observational cosmology. The department regularly obtains allocations of observing time on SERC telescopes (4.2m WHT, 3.9m AAT and 3.8m UKIRT) and the successful applicant will exploit these facilities, often using instrumentation developed at Durham, in surveys of distant galaxies and quasars, and/or via detailed studies of galaxy populations in various environments at nearby and moderate distances. Salary is on the RA IA scale (£9,865-£15,720) according to qualifications and experience.

Informal enquiries may be made to Professor Ellis (Tel. No. (091) 374 2163).

Further Particulars may be obtained from the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE** (Tel. No. (091) 374 2263) to whom applications (three copies), including the names of three referees, should be sent not later than 28th February, 1989 (quoting ref. P2). (8296)A

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF BIOLOGY
LECTURESHIP IN PLANT PATHOLOGY

The University expects to be able to offer a permanent lectureship in plant pathology from October 1989, following the UGC initiative to create opportunities for young staff. Candidates with research experience in molecular/physiological aspects of plant pathology or in the epidemiology of plant diseases would complement the two major research divisions within the department, although other strong research interests will also be considered.

Salary will be assessed on Lecturer Grade A (£9,260-£14,500 p.a.) or B (£15,105-£19,310 p.a.).

Further particulars can be obtained from **Ms J A Doyle, Staffing Department, The University, Southampton, SO9 5NH** to whom applications (7 copies) should be returned by 28th February 1989. Please quote reference 212/JAD. (8292)A

SMITHSONIAN ASTROPHYSICAL OBSERVATORY
Submillimeter Wavelength Telescope Array Positions:
Project Director
Antenna Leader
Software and Imaging Leader
Receiver Scientist

The Smithsonian Astrophysical Observatory announces four positions associated with the design, construction, and operation of an interferometric array of submillimeter wavelength telescopes, currently in the initial stages of its design study.

The array will be a major astronomical instrument, unique in its combination of wavelength coverage (approximately 0.3-2 millimeters) and angular resolution (approximately 0.3-3 arc seconds). It will be mountain-based and is expected to take 2 years to design and 5 years to construct, at a total cost of $30 to $40 million. The Federal government has allocated support for a receiver development laboratory, starting in FY '88, and for a design study, starting in FY '89. Additional funding is being requested.

The **Project Director** will be responsible for the overall scientific technical, and administrative management of the program. The incumbent will coordinate the activities of a team of scientific and technical leaders and an administrative support staff. He/she will establish policy, prepare budget documents, and supervise hiring and other personnel matters. He/she will consult regularly with scientific, technical, and administrative advisors inside and outside the Observatory.

The Project Director must have a Ph.D. and extensive professional experience and knowledge in the techniques and practice of interferometry and/or in millimeter or submillimeter wavelength astronomy. Extensive experience in the management of significant scientific and technical enterprises, as well as excellent qualities of leadership, organization, and communication, are required.

This is a senior level position, with salary depending on qualifications and experience.

The **Antenna Leader** will report to the Project Director and will be responsible for large-scale aspects of the array, including site evaluation, selection, and development; the design, the construction, and performance of individual antennas; antenna configurations and operation as an interferometer. The incumbent will work closely with other project leaders in the scientific, technical, and receiver areas during the design and construction phases, and will have the administrative and technical support needed for this work.

The incumbent should have extensive experience in interferometry, millimeter wavelength astronomy, and/or submillimeter wavelength astronomy, as evidenced by a distinguished record of research publications, and should have a broad technical background in the construction of radiotelescopes and other instrumentation at millimeter and submillimeter wavelengths. The incumbent should have a Ph.D. in astronomy, physics, or astrophysics, as well as excellent skills in communication and organization, and the ability to work well with other scientists and with support staff in the context of a large technical project.

This is a senior level position, with salary depending upon qualifications and experience.

The **Software and Imaging Leader** will report to the Project Director and will lead the effort to define imaging requirements and develop software. Topics such as zero-spacing requirements, wide field tesselation techniques, calibration in the absence of strong continuum sources, and bandwidth synthesis will be considered in the context of designing the interferometer. In the construction phase, the incumbent will lead the software development effort, especially in the area of novel imaging algorithms, control architecture, data handling and data analysis. The incumbent will work closely with project leaders and staff in the scientific, technical, and receiver areas during the design and construction phases, and will have the needed administrative, technical, and computer support.

The incumbent should have a broad technical and scientific background in astronomical imaging problems and software development, and should have extensive experience in interferometry, millimeter wavelength astronomy, and/or submillimeter wavelength astronomy, as evidenced by a distinguished record of publications. The incumbent should have a Ph.D. in astronomy, physics, or astrophysics, as well as excellent skills in communication and organization, and should work well with other scientists and with support staff in the context of a large technical project.

This is a senior level position, with salary depending upon qualifications and experience.

The **Receiver Scientist** will report to the Receiver Leader and will work in one or several of the following areas: beam optics, SIS junction fabrication, mixer block design and matching, local oscillator generation and injection, intermediate frequency amplifiers and electronics, vacuum and refrigeration systems, analog and digital spectrometers, computer control, and software. The incumbent should have extensive experience in one or more of these areas, and should have an extensive record of both technical and astronomical publications. A Ph.D. in astronomy, physics, electrical engineering, or astrophysics is required.

The position is a 4-year term position, with salary depending upon qualifications and experience. A permanent position in this area is expected to become available at the end of the four-year term, and the incumbent would be encouraged to apply.

Each candidate should send a curriculum vitae, bibliography, statement of research interests and career goals, and the names of three professional references to: **Dr. James M. Moran, Associate Director, Smithsonian Astrophysical Observatory, 60 Garden Street, Cambridge, MA 02138, USA**, by February 28, 1989. Candidates should have each reference send a letter of recommendation to Dr. Moran by the same date. (NW3099)A

UNIVERSITY OF UMEÅ, SWEDEN

invites applications for a position as

PROFESSOR IN PLANT MOLECULAR BIOLOGY

Ref no 91

Applications are invited for a position as full professor in Plant Molecular Biology at the Department of Plant Physiology, available July 1, 1989.

Candidates should have a distinguished record of research in the area of plant molecular biology as well as proven qualities in teaching and leadership. The appointee is expected to conduct and supervise independent research and to participate in graduate teaching at the department.

Applications with curriculum vitae, copies of reprints and preprints and a statement of previous research achievements and teaching skills, all in four copies, should be sent to: University of Umeå, S-901 87 Umeå, Sweden.

For further information, write or call; Professor Gunnar Öquist, Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden. Phone 46-90-165716.

Expiring date for applications is February 24, 1989. (W5817)A

UNIVERSITY OF LIVERPOOL

LECTURESHIP IN IMMUNOLOGY

Applications are invited for the post of "New Blood" Lecturer in the Department of Immunology. The successful applicant will be expected to develop a research programme within the general area of molecular immunogenetics/immunobiology; appropriate experience would be desirable. The Department's major research interests focus on human reproductive, tumour and transplantation immunology. Informal enquiries may be made to Professor P. M. Johnson (051-709 0141, ext. 2749). Candidates will normally be 35 years or under.

Initial salary within the range £9,260–£16,345 per annum on the Lecturer A or B scales.

Applications, together with the names of three referees, should be received not later than 10th March by the **Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX**, from whom further particulars can be obtained. Quote ref. RV/245/N.

An Equal Opportunity Employer. (8267)A

SCHOOL OF PHARMACY
UNIVERSITY OF LONDON

LECTURER IN PHARMACEUTICAL CHEMISTRY

Applications are invited from candidates with research interests in synthetic or other aspects of medicinal chemistry for a Lectureship in the Department of Pharmaceutical Chemistry. The post is tenable with immediate effect and the successful candidate will be expected to contribute to both teaching and research, for which the School has excellent facilities, with opportunities for multidisciplinary and inter-departmental research.

Salary according to the scale for University Lecturers (Lecturer A £9,260–£14,500 + £1,650 L.A.; Lecturer B £15,105–£19,310 + £1,650 L.A.)

Applications, in the form of a CV, and naming three referees, should be submitted to **Mr N J Rampley, Assistant Secretary, School of Pharmacy, 29–39 Brunswick Square, London WC1N 1AX** by end of February. Previous applicants need not apply. (8287)A

The University of Wyoming
RESEARCH ASSISTANT PROFESSOR

A Research Assistant Professor position is available in the Molecular Biology Department. The successful applicant will join a group who are cloning and expressing spider silk proteins. Experience in construction and screening of cDNA libraries, Southern and Northern Blotting and recombinant protein expression desirable. Salary will depend on qualifications and be in the range of $25-30,000.

Applications with a c.v. and three letters of reference should be sent to Dr. Randolph Lewis, Box 3944 Univ. Station, Laramie, WY 82071.

The University of Wyoming is an Affirmative Action/Equal Opportunity Employer. (NW3254)A

RESEARCH FELLOW
POSITION OF EXCELLENCE

DIVISION OF TROPICAL ANIMAL PRODUCTION

TROPICAL CATTLE RESEARCH CENTRE
ROCKHAMPTON, QUEENSLAND
AUSTRALIA

$31003 - $45177

THE PROGRAM: A substantial research group has been assembled at the Tropical Cattle Research Centre to carry out gene mapping studies in cattle and sheep. The National Gene Mapping Centre is also located in Rockhampton. Current projects include defining genetic markers for specific genes, DNA finger printing and the construction of a bovine genetic map.

THE PROJECT: Recent advances permit vectors known as yeast artificial chromosomes to stably incorporate megabase fragments of DNA. This development will greatly facilitate chromosome walking and genomic cloning. The project will research megabase cloning of bovine DNA in yeast with the aim of constructing representative libraries.

THE JOB: The appointee will initiate studies on yeast cloning of bovine DNA involving the isolation of high molecular weight DNA, electrophoretic separation and cloning into yeast vectors. Interaction with other ongoing gene mapping projects will be encouraged.

THE PERSON: Applicants should have a Ph.D or equivalent qualification in molecular biology. Experience in yeast culturing and pulsed field gel electrophoresis is desirable.

CONDITIONS: The initial appointment will be for a period of three years, with Australian Government Superannuation Benefits available. Assistance with relocation will be offered.

APPLICATIONS: Stating relevant personal particulars including details of qualifications and experience, the names of at least two professional referees and quoting reference number POE88 should be directed to:

The Chief,
CSIRO Division of Tropical Animal Production,
Box 5545,
Rockhampton Mail Centre,
Queensland 4702
AUSTRALIA
Fax (079) 36 1034

CSIRO AUSTRALIA

Within four weeks of the date of this publication.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

AB1 POE88 (W5827)A

OMRF

OKLAHOMA MEDICAL RESEARCH FOUNDATION

PROTEIN CHEMISTS AND CELL BIOLOGISTS IN BLOOD RESEARCH

Research positions (postdoctoral fellow to senior research scientists) immediately available for qualified MD's/PhD's to participate in multiple NIH-funded research projects related to interaction of complement and coagulation systems in platelet activation and blood clotting. Prior training or expertise in physical biochemistry, protein chemistry and/or cell biology desired. Position for highly-motivated M.D. desiring basic or applied research training in Transfusion Medicine also available. Contact **Peter J. Sims, M.D., Ph.D., Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104.** An equal opportunity, affirmative action employer. (NW3259)A

UNIVERSITY OF READING
Postgraduate Research Institute for Sedimentology
LECTURESHIP IN PALAEOBIOLOGY

Applications are invited for the post of Lecturer in Palaeobiology with reference to Sedimentology in the newly-created Postgraduate Institute for Sedimentology from 1 September 1989. The appointee will be expected to undertake research in a palaeobiology field directly related to Sedimentology, supervise postgraduate research students, develop appropriate industrial links, and contribute to taught postgraduate and undergraduate courses. The Institute has at this stage no preference regarding the research field, but examples of topics of interest are: micropalaeontology of modern environments, palaeoecology, molecular palaeoecology, taphonomy, environmental palaeobotany, and organism-climate relationships. Initial salary up to £12760 p.a. (Lecturer Grade A) plus USS benefits. Further particulars and application forms (2 copies) available from **Personnel Office, University of Reading, Whiteknights, PO Box 217, Reading, RG6 2AH, telephoine 0734-318751.** Please quote Ref. AC.8905. Closing date 31 March 1989. (8282)A

UNIVERSITY OF CAMBRIDGE
Companion Animal Research Group, Department of Clinical Veterinary Medicine
Effects of kennelling procedures on canine behaviour and welfare
UFAW POSTDOCTORAL RESEARCH ASSISTANT (3 YEARS)

Applications are invited for the above post from persons with appropriate research experience in animal behaviour. Applicants should be under 27, and should also have a valid driving licence. Salary around £10460 pa or at appropriate point on age-related scale.

Letters of application together with cv and the names/addresses of two referees should be sent to **Dr. James Serpell, Director, Companion Animal Research Group, Sub-dept of Animal Behaviour, Madingley, Cambridge CB3 8AA.**

Closing date for applications: 15 March 1989. (8277)A

UNIVERSITY OF LONDON
Department of Thoracic Medicine, King's College and Department of Phamacology, Hunterian Institute, Royal College of Surgeons

Postdoctoral scientist required to join a team of clinicians and basic scientists to study the role of phospholipid derived mediators in acute respiratory diseases in young children. The applicant should have a PhD in Pharmacology, Physiology or Biochemistry. The post is available immediately and the person appointed will work jointly with Dr John Price at King's College Hospital and Professor Priscilla Piper at the Royal College of Surgeons, to whom informal enquiries should be made. The minimum starting salary will be £12,150 and the post is funded intitially for 2 years.

Applicants should send a CV and the names and addresses of two refrees as soon as possible to:—

Personnel Officer, Royal College of Surgeons, 35-43 Lincoln's Inn Fields, London WC2 3PN. (8275)A

ansto

AUSTRALIAN NUCLEAR SCIENCE & TECHNOLOGY ORGANISATION

RESEARCH FELLOW
MATERIALS SCIENCE/ELECTRON MICROSCOPY

Position No. 762

Research Scientist
$A31,003-$A38,045 per annum

Senior Research Scientist
$A39,349-$A45,177 per annum

A vacancy currently exists within the Advanced Materials Program of the Australian Nuclear Science and Technology Organisation (ANSTO) for a research fellowship within the Electron Microscopy Group. The successful applicant will be expected to interact fully with other scientists in the program and will have opportunity to participate in collaborative programs with industry. Current projects include:

- Phase development and crystal chemistry of Synroc, a titanate ceramic for nuclear waste encapsulation.
- Studies of structural changes in minerals and synroc during weathering or hydrothermal dissolution.
- Radiation damage in metamict minerals and neutron irradiated ceramics.
- Advanced ceramics for biomedical and electrical applications e.g. high Tc superconductors, proton conductors etc.
- Remnant life studies of steels.

The fellow will also be encouraged to develop his/her own research projects and/or industrial collaboration, provided such interests fall within the Programs general aims.

The Advanced Materials Program has one JEM 2000 FX TEM fitted with a Gatan TV system and a conventional Si/Li ED spectrometer; and a second JEM 2000 FX fitted with a wide bore pole piece and a thin window detector. Other facilities include a JEOL JSM 6400 SEM, a JEOL JXA 840 analytical SEM, a computer controlled Phillips PW 1050 diffractometer, a Siemens D 500 automatic XRD unit, optical microscopes, a Zeiss quantitative image analysis system, mechanical testing equipment and an extensive ceramics processing laboratory.

The Organisation utilises a salary system based on personal merit for this category of staff. Progression to higher grades is possible as experience, skills, knowledge and personal contributions to the Organisation's programs increase.

Salary depending upon qualifications and experience will be within the above range.

Knowledge, qualifications and Skills
Applications are sought from electron microscopists **and** from materials scientists with experience in analytical TEM techniques. The skills sought include experience with HRTEM, ATEM, image processing, image simulation, materials fabrication, metallurgy, crystal chemistry, mineral dissolution/alteration, structure/property relationships, radiation damage, and electroceramics.

Applicants should have a PhD or equivalent in relevant physical sciences and extensive experience with transmission electron microscopy.

At least two Professional referees should be nominated. It is intended that the skills and experience of the successful applicant will complement those of existing staff.

Employment will be for a term of 3 years, with Australian Government superannuation benefits available.

Contact Officer for further information is Dr K Smith telephone (02) 543 3505 or Fax (02) 543 5097.

Closing date for applications is 17 February, 1989, although applications received after that date may be considered. It is hoped that the successful applicant would begin duties on or before 1/4/89.

Application forms and selection criteria may be obtained by telephoning (02) 543 3064 or writing to the Recruitment Officer, Australian Nuclear Science and Technology Organisation, Private Mail Bag 1, Menai, N.S.W. 2234, Australia, quoting the above position number.

ANSTO IS AN EQUAL OPPORTUNITY EMPLOYER.

(W5775)A

BMFT – Förderprogramm Neurobiologie

Das Bundesministerium für Forschung und Technologie beabsichtigt eine Förderung besonders ausgesuchter Projekte der neurobiologischen Forschung (*Beginn: ca. 1. Sept. 1989, Laufzeit: 5 Jahre*). In Frage kommen Projekte aus den Bereichen der molekularen und zellulären Neurobiologie sowie der funktionellen Analyse des Gehirns. Der Leiter einer Projektgruppe kann seine eigene Stelle mitbeantragen. Um die Einrichtung einer Projektgruppe können sich Institutionen oder entsprechend qualifizierte Wissenschaftler in Verbindung mit Institutionen bewerben. Einzelheiten zum Förderprogramm und zur Abfassung von Projektskizzen können unter dem Stichwort "Projektgruppe Neurobiologie" über die Zuständige Stelle:

Naturwissenschaftliches und Medizinisches Institut
an der Universität Tübingen in Reutlingen (NMI)
Gustav-Werner-Str. 3, D–7410 Reutlingen 1, F.R.G.

in Erfahrung gebracht werden. Projektskizzen sind bis zum 15. April 1989 ebenfalls beim NMI einzureichen. (W5810)A

HUMAN GENETICIST

The Department of Molecular Genetics, Biochemistry and Microbiology is searching for a human geneticist particularly an individual who is interested in genetic diseases effecting the cardiovascular or pulmonary system. The appointment is in the tenure tract and can be at any level. Generous space and start up monies are available. In addition, the College has been awarded a Program of Excellence for the Heart, Blood, and Lung Institute. This grant provides supplies and postdoctoral fellow support for this position over the 7 years of the award. The current department has 26 faculty and 50 graduate students and maintains core facilities in tissue culture, DNA technology and transgenic mice.

Interested applicants should submit their curriculum vitae, a short write up of their anticipated research, and names of three individuals who are qualified to judge their abilities to: **Jerry B Lingrel, PhD., Professor and Chair, Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267-0524.**

The University of Cincinnati is an Equal Opportunity/Affirmative Action Employer (NW3258)A

NATIONAL INSTITUTE FO
BIOLOGICAL STANDARI
AND CONTROL
TECHNICIAN
COLLABORATING CENTRE
ON AIDS

A technician is required to assis investigations into the molecu biology of simian retroviruses.

The successful applicant will involved in the cloning sequenc and expression of simian retro DNA and its amplification by polymerase chain reaction (PC

Applicants should possess degree or equivalent in a l logical science, preferably w relevant post graduate experien though full training will be p vided.

Informal enquiries can be m to Dr Neil Almond Ext 439.

Salary is on a scale up to £10, per annum dependent on qualifications and experience.

For further details and an ap cation form please apply to Personnel Office, Blanche La South Mimms, Herts. EN6 3C Telephone 0707 54753 Ext 457.

Please quote reference num SU/AL/360.

Closing date for applications February 1989. (8283)A

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
Department of Neuroscience

LECTURESHIP IN MOLECULAR BIOLOGY

Applicants are invited for the above post. Applicants should have molecular biological expertise and be interested in applying molecular biology to study the nervous system although previous neuroscience expertise is not necessary.

Salary on the non-clinical lecturer scale. Starting salary according to age and experience.

Applications in the form of a curriculum vitae plus the names of two referees should be sent to the **Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF** from whom further information may be obtained.

Closing date 23 February 1989. (8263)A

UNIVERSITY OF READING
DEPARTMENT OF CHEMISTRY
Applications are invited for a

LECTURESHIP IN INORGANIC CHEMISTRY

from 1 September 1989. Preference will be given to candidates who have an interest in the synthesis of inorganic materials and their characterisation and applications Salary scale £9260 to £14500 p.a. (Grade A) or £15105 to £19310 p.a. (Grade B) plus USS benefits.

Further particulars and application forms (2 copies) are available from the **Personnel Officer, University o Reading, Whiteknights, PO Box 217, Reading, RG6 2AH telephone (0734) 318751.** Closing date 31 March 1989 Please quote Ref. AC.8904. (8265)A

University of Bristol
Department of Animal Husbandry

POST DOCTORAL MICROBIOLOGIST

Required to study the fate of pathogenic bacteria following aerosolisation and inhalation using both *in vivo* and *in vitro* techniques. Applicants should be qualified in microbiology and have interests in infectious desease and aerobiology. The post is funded for three years.

Salary is dependent upon age and experience within the range 1A £9865-£15720 pa.

For further details telephone Bristol 303136 (ansaphone after 5pm) or write to the **Personnel Office, Senate House, Bristol BS8 1TH.** Please quote reference A164.

Closing date 17 February 1989.

An Equal Opportunities Employer. (8266)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

RESEARCH ASSISTANT

Applications are invited for a research assistant to investigate senile cataract i the human. The project will involve the use of electron microscopy, intracellula microelectrode recording and vital staining to examine the structure/functio relationship of the lens epithelium and investigate how this breaks down in th pathology of senile cataract.

Applicants should have a good honours degree in a relevant subject and expe ience in tissue culture and electron microscopy would be preferable.

This post is for 3 years and is funded by Research into Ageing. Salary in th range £10,325-£13,330 including London Weighting on the scale R1B f Research and Analogous Staff.

Further particulars and an application form from the **Personnel Office St George's Hospital Medical School, Cranmer Terrace, London, SW17 0R Telephone 01-672 9944 ext 56020.** Closing date 24 February 1989. Please quo reference 8/89. (8259)A

ETH ZÜRICH

ostdoctoral Position
TH-Zürich, Switzerland
ilable to study the molecular basis of **omosome- and chromatin structures** east with respect to gene expression, ification, and DNA repair (Thoma & chej, 1988, Cell 55, Dec. 23). The posi- is available for 3 years starting April 9. The salary will be US$ 38000 to 00 (Swiss National Science Founda- n) depending on experience.
e candidate should have competence yeast genetics and/or molecular bio- y and/or immunological techniques. ase call or send application to: **Dr. tz Thoma, Institut für Zellbiologie, H-Hönggerberg, CH-8093 Zürich, itzerland. Tel: 0041-1-3773323 or 41-1-3773352.** (W5793)A

UNIVERSITY OF EDINBURGH
POSTDOCTORAL RESEARCH FELLOW
DEPARTMENT OF VETERINARY CLINICAL STUDIES

pplications are invited for a ree-year, extramurally funded search post involving mast cell roteinases, their isolation, haracterisation and functional roperties.

he post is available from 1st ebruary 1989. Salary is £9,865 .a. rising to £10,460 in the econd year and £11,070 in the hird year.

urther particulars may be btained from the **Personnel ffice University of Edinburgh, 3 South Bridge, Edinburgh, H1 1LS,** with who applications, ncluding a *curriculum vitae* and he names and addresses of two eferees, should be lodged not ater than 17th February 1989.

lease quote reference No. 5630
(8269)A

School for Field Studies

School for Field Studies seeks Sr. & Assoc. faculty for summer programs: Ethnobot./Ecuador; Landscape Ecol./Colorado; Tropical Ecol./Costa Rica; Acid Rain/Adirondacks; Wildlife Mngt./Kenya; Cult. Ecol./Himalayas; Bald Eagles/Alaska; Coral Reef Ecol. & Marine Pks. Mngt./Virgin Isles; Monkseals/Greece; Spinner Dolphins/Hawaii; Humpback Whale Ecol./N. Atlantic; Dolphins/Alabama. Ph.D. (MS. for Assoc. Fac.), excel. teaching skills, field resch. exp., enthus. for experien- ial ed. Send detailed letter, cv, and refs. to the **Dean, SFS, 16 Broadway, Beverly, MA 01915 (508) 927-7777.** (NW3203)A

Natural Sciences and Engineering Research Council of Canada

Conseil de recherches en sciences naturelles et en génie du Canada

REGISTER OF CANADIANS STUDYING ABROAD

The Natural Sciences and Engineering Research Council of Canada (NSERC) has recently initiated a Register of Canadian postgraduate students and postdoctoral fellows in the fields of natural science or engineering who are currently pursuing studies or research abroad. The object of the Register is to facilitate their return to Canada by providing a means of contact with potential Canadian employers.

Eligibiligy: Canadian citizens and permanent residents of Canada enrolled in a postgraduate degree program or holding a postdoctoral appointment at a non-Canadian university/institute, in the natural sciences or engineering, are eligible to register.

Operation: In conjunction with the Register of students and fellows, NSERC will maintain a list of interested Canadian employers in industries, universities, and government departments. Names and contact addresses of registrants meeting specified qualifications will be provided on request to these employers.

NSERC will provide initial contact between registrants and potential employers; further contact will be the responsibility of the employers. This service is offered free of charge to registrants and potential employers.

Registration: Canadian students and fellows interested in obtaining further information and/or registration forms should contact:

Administrator, Register of Canadians Studying Abroad
Natural Sciences and Engineering Research Council of Canada
200 Kent Street
Ottawa, Ontario K1A 1H5
Canada
Telephone: (613) 995-6060 (W5815)A

Canada

nature

— the world's most prestigious weekly journal of science

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
RESEARCH TECHNICIAN

required to join a team supported by the MRC/Government AIDS Directed Programme to develop laboratory tests for vaccine efficacy. Virological experience clearly an advantage, but work mostly cellular immunology for which training will be given.

Salary in the range £6,636 – £7,828 plus London Allowance per annum.

Further details and an application form from the **Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Tel 01-672 9944 (ext 56020). Closing date Friday 17 February 1989. Please quote reference no. 15/89.** (8276)A

THE UNIVERSITY OF NEW ENGLAND

Equal Opportunity is University Policy

DEPARTMENT OF BIOCHEMISTRY, MICROBIOLOGY AND NUTRITION

RESEARCH FELLOW – ACIAR

(Re-advertisement)

The position of Research Fellow is funded by the Australian Centre of International Agricultural Research. The successful applicant will be expected to organise and collaborate in research into increasing livestock production from crop residues and to spend periods of each year in India at the Central Institute for Research in Buffaloes, Hisar, or at the National Dairy Development Board, Anand, collaborating with teams of animal scientists. Presently research is underway on improving the nutritional value of straw as a ruminant feed by ammoniation, supplementation and defaunation of the rumen of cattle of straw-based diets.

The applicant should have a background in agricultural science or veterinary science, a higher degree or equivalent qualification. Experience in developing countries would be an advantage but not essential. The appointment will be on an annual basis but funds are available for 3 years in the first instance.

Informal enquiries to Professor R.A. Leng, telephone 61 67 73 2707.

SALARY: $A30,737-$A40,100 per annum.

CLOSING DATE: 17th February, 1989.

POSITION No.: 419.

RESEARCH FELLOW

RESEARCH FELLOW
Enzymologist

(Re-advertisement)

The successful candidate will join a team of microbiologists, molecular biologists and animal nutritionists, investigating means of increasing fibre digestion in the rumen of sheep and cattle.

The enzymologist would be required to investigate the enzymes and rate-limiting steps involved in cellulose breakdown by rumen bacteria. Experience in anaerobic microbiology would be an advantage. The appointment would be for one year in the first instance. The project is funded by the Australian Meat and Livestock Research and Development Committee. The project will be reviewed in 1990.

ENQUIRIES: Dr K. Gregg 61 67 73 2748.

SALARY: $A30,737-$A40,100 per annum.

CLOSING DATE: 17th February, 1989.

POSITION No.: 420.

JUNIOR RESEARCH FELLOW

Microbiologist

(Re-advertisement)

Applications are invited for a grant-funded scientific post tenable in the Department of Biochemistry, Microbiology and Nutrition. The successful candidate will join a team of anaerobic microbiologists, molecular biologists and animal nutritionists, investigating means of increasing fibre digestion in the rumen of sheep and cattle.

The bacteriologist would be required to investigate and compare fibre-digesting bacteria present in the gut of herbivorous animals. Experience in anaerobic microbiology would be an advantage but training can be given. The appointment would be for one year in the first instance.

ENQUIRIES: Dr T. Bauchop 61 67 73 2606.

SALARY: $A22,109-$A25,867 per annum (Grade I).
$A26,617-$A30,360 per annum (Grade II).

CLOSING DATE: 17th February, 1989.

POSITION No.: 421.

JUNIOR RESEARCH FELLOW

Ruminant Nutritionist

(Re-advertisement)

A Junior Research Fellow is required to be part of a research team aiming to improve the efficiency of ruminant animal production through selection or genetic modification of rumen micro-organisms. The research is sponsored by the Australian Meat and Livestock Research and Development Corporation.

The successful applicant must have a PhD in the area of ruminant nutrition and metabolism, and expertise with isotope tracer methodology and kinetic modelling would be an advantage.

ENQUIRIES: Dr J.V. Nolan 61 67 73 2605.

SALARY: $A22,109-$A25,867 per annum (Grade I).
$A26,617-$A30,360 per annum (Grade II).

CLOSING DATE: 17th February, 1989.

POSITION No.: 422.

Applications for the above positions should be addressed to the Staff Officer and include names and addresses of three referees and state position number. Referees should be given a copy of this advertisement and asked to write direct to the Staff Officer by the closing dates and quote position number.

ARMIDALE NSW 2351
AUSTRALIA

(W5824)A

UNIVERSITY OF OXFORD

In association with Magdalen College

UNIVERSITY LECTURERSHIP IN CHEMICAL PATHOLOGY

Applications are invited for the above post tenable from 1 October 1989. Candidates will have specialised in molecular biology with a strong interest in the application of recombinant DNA technology to medical problems.

The holder of this post will be expected to contribute to advanced teaching in Cell Biology.

The ability to make some contribution to introductory teaching in Pathology, Bacteriology or Virology would be an advantage. Stipend according to age on the scale £9,865 to £20,615.

The successful candidate may be offered a tutorial fellowship at Magdalen College.

Further details may be obtained from

Professor H. Harris, FRS,
Sir William Dunn School of Pathology,
South Parks Road,
Oxford, OX1 3RE,

to whom applications (nine typed copies or one from overseas applicants) with full curriculum vitae, summary of research interests and the names of three referees should be sent by 13 March 1989. (8293)A

The University is an Equal Opportunity Employer

SCOTTISH CROP RESEARCH INSTITUTE

Invergowrie, Dundee DD2 5DA

MOLECULAR BIOLOGIST

HIGHER SCIENTIFIC OFFICER (BAND I) (3-year Appointment) VIROLOGY DEPARTMENT

An HSO is required to study parsnip yellow fleck virus, a novel plai virus which resembles the picornairuses of animals. The objective to determine the nucleotide sequence and gene products of the viru its affinities with other viruses, and the expression of its RNA in plai protoplasts.

Salary scale: £10,026–£13,460 plus
Non-contributory superannuation scheme.

Qualifications: First or upper second class honours degree in a appropriate priate subject, with at least two years' relevant pos graduate research experience, which may be a course of research an study leading to a Ph.D. degree.

The Institute is an equal opportunities employer.

Further paricular may be obtained from Mr I. Paxton, Personn Officer (tel: 0382 562731), to whom a curriculum vitae with th names and addresses of three referees should be sent by Friday 1 February 1989. (8280)A

POSTDOCTORAL POSITION
Molecular Neurobiology

A postdoctoral position is available to analyze the molecula characteristics of cell surface glycoproteins and their role i intercellular communication in the nervous system. Project concern the interaction of contactin (J.Cell Biol. [1988 107:1561-1573) with extracellular and intracellular ligand and the molecular analysis, expression and role of molecule isolated in association with contactin. Candidates witl expertise in biochemistry, molecular biology, cell-, develop mental-, or neurobiology may reply. The La Jolla Cance Research Foundation is located within the scientific commu nity of San Diego.

To apply please submit curriculum vitae, statement o research interests and two letters of recommendation to:

Dr. Barbara Ranscht, La Jolla Cancer Research Foundation 10901 N. Torrey Pines Rd., La Jolla, CA 92037.

An Equal Opportunity/Affirmative Action Employer.

(NW3250)A

RESEARCH OPPORTUNITY IN JAPAN

The Frontier Research Program at the **RIKEN** Institute of Physical and Chemical Research (see Nature 325: 569, 1987) invites applications for postdoctoral positions in the following areas:

— Molecular biology of the immune system and protein chemistry (purification and analysis of proteins) including expression of cloned genes and characterization of gene products at the Laboratory for Molecular Regulation of Aging headed by *Dr. Gabriel Gachelin.*

— Molecular biology of tumor suppresser genes, protein chemistry including characterization of proteins from the brains of Alzheimer patients, and biochemistry, molecular cell biology including eukaryotic cell during proliferation and differentiation at the Laboratory for the Aging Process headed by *Dr. Koiti Titani.*

— Molecular biology of phytochrome, including immunocytochemistry, isolation and analysis of genomic clones *in vivo* footprint analysis and site-directed mutagenetics at the Laboratory for Plant Biological Regulation headed by *Professor Masaki Furuya.*

— Nonlinear optics of organic materials, characterization and device application and organic molecular beam epitaxy technique at the Laboratory for Nonlinear Optics and Advanced Materials headed by *Professor Anthony F. Garito.*

— Protein design and synthesis, and bioelectronics at the Laboratory for the Bioelectronic Materials headed by *Dr. Kevin M. Ulmer.*

Appointments will be initially for one year and may be renewable thereafter.

These Laboratories are located at RIKEN Institute's two establishments in Wako, Saitama and Tsukuba, Ibaraki, on the outskirts of Tokyo.

Candidates should send a detailed curriculum vitae, a list of publications and the names of two individuals who may be contacted for references and summary of research interests to:
Director, Division of the Frontier Research Program,
RIKEN, Wako, Saitama, Japan
Zip code 351–01

(W5785)A

UNIVERSITY OF LIVERPOOL
Department of Haematology
Postgraduate Research Assistant

Applications are invited for the post of Research Assistant to work on a project investigating the role of specific growth factors in the growth and differentiation of normal and leukaemic human bone marrow in culture. A good honours degree in a related subject is required and some experience of tissue culture, an advantage. The post is for one year in the first instance but is probably renewable for a further two years. Informal enquiries may be made either to Professor J.C. Cawley (051-709 0141, ext. 2811) or Dr. J.A. Carron (051-709 0141, ext. 2815).

Initial salary on the scale £8,675-£11,680 per annum.

Applications, together with the names of three referees, should be received not later than 20 February 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3BX,** from whom further particulars may be obtained.

Quote ref. RV/246/N

An Equal Opportunity Employer (8268)A

AGRICULTURAL & FOOD RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY & GENETICS RESEARCH

ABRAHAM, CAMBRIDGE CB2 4AT

IMMUNOLOGICAL REGULATION OF ANIMAL GROWTH

POST-DOCTORAL POSITION available in the Department of Immunology, to investigate growth promotion in rodents and farm animals by immunological methods. The position, which is commercially funded, is available for two years in the first instance with possibility of renewal. Previous experience in immunology, biochemistry or growth regulation an advantage.

Appointment in Higher Scientific Officer grade. Salary £10,026 to £13,460 according to qualifications and experience. 25 days' leave and 10.5 public holidays a year.

Further details from Dr M Taussig or Dr G Butcher (telephone (0223) 832312). Application form available from the Personnel Office quoting Ref: II/ST by 21 February 1989. (8310)A

UNIVERSITY OF
essex

SIGNAL TRANSDUCTION IN HUMAN NEUTROPHILS
Department of Chemistry and Biological Chemistry

Applications are invited for the position of Senior Research Officer to work on **signal transduction mechanisms to the cytoskeleton in human neutrophils** with Dr Paul Thornalley. The project is based on the novel and exciting prospect of involvement of the glyoxalase system in cytoskeletal regulation (Biochim. Biophys. Acta **931**, 120, 1987) and involves immunofluorescence microscopy with digital image processing, phosphoprotein technology employed in studies of chemotaxis, phagocytosis and degranulation of human neutrophils. Applicants should have interests in biochemistry and/or cell biology and enjoy working in a multi-disciplinary life sciences laboratory.

The successful applicant can expect to join a lively and enthusiastic research group and contribute to on-going international collaborations. The post is funded by the Wellcome Trust. The appointment will be for three years with salary on the research assistant Grade 1A scale (£9,865 - 15,720).

Informal enquiries may be made to Paul Thornalley (Tel. 0206 873010 direct dial) and further particulars obtained from the Personnel Office (Tel. 0206 873433).

Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (R/788/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ by 28th February 1989.

(8318)A

HYPOGUARD (UK) LTD

Manufacturers of Diabetic Equipment require

LABORATORY TECHNICIAN

To assist in the operation of the chemical coating laboratory in the Diagnostics Division.

Duties to include:

Preparation of stock production chemicals, overseeing production and QC systems and personnel supervision. Some new product R+D is also envisaged.

Suitable applicant will probably hold HNC/HTEC in physical chemistry or chemistry, be capable of working in a supervisory capacity to production staff, and be capable of working on own initiative when necessary.

Salary to £10,000, depending on age, qualifications and aptitude.

Applications in writing including CV, or requests for further information to:

STEPHEN GULLICK
PROJECT MANAGER
Hypoguard (UK) Ltd
Dock Lane
Melton
Woodbridge
Suffolk
IP12 1PE

(8314)A

UNIVERSITY OF

essex

DEVELOPMENT OF NOVEL ANTI-CANCER DRUGS

Department of Chemistry and Biological Chemistry

Applications are invited for the position of Research Officer, Grade IB, to work on the development of **anti-mitotic agents for cancer chemotherapy, based on the novel and exciting prospect of anti-mitotic activity of S-acylglutathione derivatives.** The project involves synthesis of modified tripeptides, enzyme purification and kinetic studies, and culture of human leukaemia cells. Applicants should have interests in any of peptide synthesis, enzymology and cell culture and enjoy working in a multidisciplinary life sciences laboratory.

The successful applicant can expect to join a lively, enthusiastic and expanding research group working on related subjects and contribute to international collaborations. The post is funded by the Leukaemia Research Fund. Appointment will be for three years with salary on the research assistant IB scale (£8,675 - 11,680), with the opportunity of registering for a higher degree.

Informal enquiries should be made to Paul Thornalley (Tel. 0206 873010) and further particulars obtained from the Personnel Office (Tel. 0206 873433).

Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (R/809/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ by 28th February 1989.

(8319)A

MOLECULAR BIOLOGIST
DEPARTMENT OF DERMATOLOGY
STANFORD UNIVERSITY SCHOOL OF MEDICINE

The Department of Dermatology of Stanford University School of Medicine invites nominations and applications of individuals possessing scientific expertise in the molecular biology of the extracellular matrix for a tenure-track position on its faculty. Candidates should hold the M.D. or Ph.D. degree and present strong evidence of research accomplishments. Applicants should also be committed to the training of post-doctoral fellows in cutaneous biology. The rank of the appointed person will be commensurate with academic qualifications. Stanford University is committed to increasing the representation of women and members of minority groups on its faculty and encourages applications from such candidates. Interested individuals should submit a curriculum vitae and direct correspondence to:

Eugene A. Bauer, M.D.
Professor and Chairman
Department of Dermatology, R–144
Stanford University School of Medicine
Stanford, California 94305

(NW3267)A

RESEARCH FELLOW

(SATELLITE INSTRUMENTATION AND REMOTE SENSING)

$A31,003 - $45,177

DIVISION OF ATMOSPHERIC RESEARCH
ASPENDALE, VICTORIA, AUSTRALIA

THE DIVISION: The Division of Atmospheric Research conducts strategic and applied research into problems concerning the physics, dynamics and chemistry of the atmosphere.

THE PROGRAM: The Remote Sensing Group is concerned with remote sensing of atmospheric and surface parameters. Projects include: the interpretation and application of high resolution meteorological satellite data, the investigation of clouds, aerosols and plumes by lidar; and theoretical and experimental investigation of candidate instrumentation for future meteorological satellites.

A numerical model and a laboratory prototype of an instrument to measure surface pressure from space has been investigated. An airborne prototype is under construction, and test flights on CSIRO's research aircraft are scheduled for July - December 1989.

THE JOB: Initially, to undertake research into the development of the atmospheric pressure sensor and the interpretation of data from the test flights. Thereafter the appointee will have considerable freedom to initiate research within the general field.

THE PERSON: Applicants should have a Ph.D degree or equivalent qualifications in physics together with proven research ability. Experience in general meteorology and, in particular, the theory of radiative transfer would be an advantage.

CONDITIONS: Appointment will be for a term of 3 years. Australian Government superannuation benefits are available.

MORE INFORMATION: Prospective applicants are invited to telephone Dr. Denis O'Brien on 0011 613 586 7662 for further information. Dr. O'Brien can also provide a copy of the detailed job description and selection criteria.

APPLICATIONS: Applications should be submitted by 3 March 1989 and quote Reference Number A1845. They should be framed against the selection criteria, and should state relevant personal particulars including details of qualifications and experience. Applicants should nominate at least two professional referees, and address their application to:

The Chief,
CSIRO Division of
Atmospheric Research,
Private Bag No. 1,
Mordialloc, Vic.,
Australia, 3195.

CSIRO AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER.

(NW3299)A

UNIVERSITY OF OSLO

TWO PROFESSORSHIPS IN GENERAL GENETICS

re vacant at the University of Oslo. Both professorships come un-
er the Faculty of Mathematics and Natural Sciences and are placed
ithin the Department of Biology:

. **Professorship-(salary class 35) in General Genetics (Population Genetics),** vacant from 1 January 1990.

he University is looking for a person with experience from both
eoretical and empirical aspects of Population Genetics and with
trong background in classical Genetics. Preference will be given to
andidates who are using modern techniques, such as DNA-hybridi-
ising, DNA-fingerprinting etc.

he salary payable in class 35 will be NOK 269 710 gross, from which
% will be deducted to cover contributions to the Norwegian Public
ervice Pension Fund.

. **Professorship (salary class 33) in General Genetics (Molecular Genetics),** vacant from 1 January 1989.

he University is interested in applicants with scientific competence
molecular eukaryot Genetics; applicants who have worked with
ie genetics of procaryots and virus will also be seriously evaluated.

he salary payable in class 33 will be NOK 248 757 gross, from which
% will be deducted to cover contributions to the Norwegian Public
ervice Pension Fund.

or both A and B:

urther details concerning the field of study and the responsibility
f the professor, and a description of the working conditions in the
epartment of Biology have been included in a separate statement
hich may be obtained on application to the Secretariat of the
aculty of Mathematics and Natural Sciences, PO Box 1032
lindern, N-0315 Oslo 3, Norway.

he professor will be appointed to participate in teaching and exa-
inations in accordance with the academic regulations in force, and
e or she will be expected to accept, without indemnity, any changes
iat may be made with respect to curriculum, pension scheme and
ompulsory retirement age by Act or by the King with the consent
f the Storting.

/ithin one month of the closing date for applications, candidates
ust send direct to the Secretariat of the Faculty of Mathematics
id Natural Sciences of the University of Oslo:

5 complete sets of the academic works — published or unpublished — that they wish to have taken into account by details of where they have been made public, and 5 copies of the application with any enclosures.

cademic work in progress at the time of the closing date for appli-
itions may however be submitted up to three months after the clo-
ng date, provided the applicant has given notice of this at the time
submitting the other works.

fter the closing date for applications, the University wi'i send
ndidates information concerning the submission of their academic
ork.

eference is also made to the Rules concerning the Procedure for the
ppointment of Professors and Readers, laid down by the Senate,
f the University Act § 18.

pplicants must be able to produce a medical certificate on request.

several applicants are considered to have equal qualifications
ter both the academic/professional and the pedagogical qualifica-
ns have been assessed, the University will rank a female appli-
nt before a male applicant.

pplications are to be addressed to the King and sent with one copy
the list of academic works to **the University of Oslo,** PO Box 1072
lindern, N-0316 Oslo 3, Norway by 1 April 1989.

(W5813)A

nature

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THE UNIVERSITY OF LIVERPOOL

MEDICAL BIOLOGICAL SCIENCES

POSTGRADUATE RESEARCH OPPORTUNITIES

The School of Medical Biological Sciences comprises the Departments of Human Anatomy and Cell Biology, Physiology, and Pharmacology and Therapeutics. A number of Studentships will be available for research leading to a Ph.D. in the following areas:

Department of Human Anatomy and Cell Biology

1. The role of rigor complexes in anoxic injury of cardiomyocytes (Dr. P. H. Cobbold).
2. The function of specific actin sequences in the dynamics of actin architectures. (Dr. P. Sheterline).
3. *In situ* hybridisation study of transforming skeletal muscle. (Prof. S. Salmons).
4. Proto-oncogene expression in mitogenesis. (Dr. S. Pennington).
5. The mechanism of the calcium signal in mouse oocytes at fertilisation. (Dr. K. S. R. Cuthbertson).
6. Transplantation of foetal neural tissues and neuronal cell lines into mammalian brain. (Dr. Y. S. Allen).
7. Role of plasminogen activator in human bone remodelling. (Dr. J. A. Gallagher).
8. Laminin expression in the nervous system. (Dr. D. Edgar).

Contact: Dr. P. H. Cobbold
Tel: (051) 794 5470

Department of Physiology

9. Ion channels in secretory cells. (Prof. O. H. Petersen).
10. Biosynthesis of brain-gut peptides. (Prof. G. J. Dockray).
11. Control of exocytosis in secretory cells. (Dr. R. D. Burgoyne).
12. Central nervous regulation of the upper gastrointestinal tract. (Dr. R. J. Gayton).
13. The somatosensory input to the rat striatum. (Dr. D. C. M. Taylor).
14. Excitatory amino acid neurotransmitters. (Dr. J. Garthwaite).
15. Structure-activity relationships of regulatory peptides. (Dr. R. Dimaline).
16. Regulation of calcium release and calcium influx in exocrine acinar cells. (Dr. D. V. Gallacher).

Contact: Dr. R. D. Burgoyne
Tel: (051) 794 5305

Department of Pharmacology and Therapeutics

17. Metabolism of cyclosporin. (Dr. D. J. Back).
18. Blood-borne factors in myocardial ischaemia. (Dr. S. J. Coker).
19. Control of mast cell sensitization. (Dr. J. W. Coleman).
20. Pharmacological and parasitological studies of antimalarial agents derived from *Artemesia annua*. (Dr. I. G. Edwards).
21. Effect of drug binding inhibitors on anion transport by the kidney. (Dr. W. E. Lindup).
22. Retrograde axonal transport of specific proteins in peripheral neuropathy. (Dr. W. G. McLean).
23. Effect of chemical modification on drug toxicity using isolated human enzymes and target cells. (Dr. B. K. Park).
24. Effect of thyroid dysfunction on the multiple forms of UDP-glucuronyl transferase in man and the rat. (Dr. A. K. Scott).

Contact: Dr. W. E. Lindup
Tel: (051) 794 5556

Further information may be obtained by writing to the named contacts at **The University of Liverpool, P.O. Box 147, Liverpool L69 3BX,** enclosing a curriculum vitae with the names and addresses of two referees, and indicating the research area(s) of interest. Candidates wishing to be considered for a Wellcome Trust Prize Studentship should apply as soon as possible.

(8291)A

UNIVERSITY of LAUSANNE

Our Institut de Police scientifique et de Criminologie has an opening for an

Associate Professor in Forensic Science

The position entails teaching, academic research, R & D and consultancy at local and international level. The candidate must be capable of fitting into a small, dedicated team (2 professors, 10 scientific and technical assistants).

The fields of interest of the candidate may be diverse (methodology, chemical criminalistics, firearms identification, arson, explosion, questioned document examination, computer security, drugs of abuse and drug intelligence, forensic biology exluding serology). Twelve months after appointment, the teaching should be given proficiently in french.

Candidates should send their curriculum vitae to the Dean, Faculté de Droit, BFSH 1, 1015 Lausanne, Switzerland, by March 31, 1989.

For information contact:

Professor Pierre Margot — Institut de Police scienfifique et de criminologie — Place du Château 3 — CH — 1005 Lausanne. (W5830)A

RESEARCH FELLOW
BIOSPHERE MODELLER

$A31,003 - $45,177

DIVISION OF ATMOSPHERIC RESEARCH
ASPENDALE, VICTORIA, AUSTRALIA

THE DIVISION: The Division of Atmospheric Research conducts strategic and applied research into problems concerning the physics, dynamics and chemistry of the atmosphere.

THE PROGRAM: The Global Atmospheric Change program aims to predict and understand the mechanisms responsible for changes in the chemical and physical character of the regional and global atmosphere, particularly where these changes may affect the Australian community. One project is concerned with generating realistic scenarios and predictions of local and regional climates under conditions of changing global climate, and to estimate the impact of such climates on the biosphere and human activity in collaboration with the International Geosphere-Biosphere program.

THE JOB: To develop climate-biosphere interface models at two levels: (i) simple biosphere models capable of responding to climate change and increasing CO_2 concentrations, which will provide appropriate changing lower boundary conditions for climate models, and (ii) crop or ecosystem models capable of describing the changes in biospheric productivity and composition which would result from climatic change.

THE PERSON: Applicants should have a Ph.D. or equivalent qualifications in plant biology/physiology and/or ecology, with a strong interest in mathematics or the physical sciences; or a Ph.D. or equivalent qualifications in mathematics/atmospheric sciences with a strong interest in plant biology/physiology and/or ecology. Experience in computer modelling is also essential.

CONDITIONS: The appointment is for a term of three years. Australian Government superannuation benefits are available.

MORE INFORMATION: Prospective applicants are invited to contact Dr. Barrie Pittock on (0011 61 3) 586 7527, Fax 586 7600, for further information. Dr. Pittock can also provide a copy of the duty statement and selection criteria.

APPLICATIONS: Applications should be submitted by 3 March 1989 and quote reference number M8959. They should be framed against the selection criteria and should provide details of relevant personal particulars, including qualifications and experience. Applicants should nominate at least two professional referees, and address their applications to:

The Chief,
CSIRO Division of Atmospheric Research
Private Bag No. 1
Mordialloc, Victoria,
Australia, 3195

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

AT89/04a

DEAN OF THE COLLEGE OF BIOLOGICAL SCIENCES
THE OHIO STATE UNIVERSITY

Applications and nominations are invited for the position of Dean of Biological Sciences. As chief executive officer, the Dean is responsible for academic leadership, resources management, and productive synergistic relationships with other Colleges in the University including Agriculture, Mathematical and Physical Sciences, Medicine, Pharmacy and the Biotechnology Center. The Dean also plays a central role in a range of development and public relations activities. Candidates should have an earned Ph.D. degree and a record of research accomplishment that warrants a faculty appointment at the rank of full Professor in the College. The deanship is a twelve-month appointment available September 1, 1989. Salary is negotiable and commensurate with background and experience. Nominations or letters or application, curricula vitae, and names, addresses, and telephone numbers of four references should be addressed to:

Dean C. William Kern, Chair, Biological Sciences Search Committee, Office of Academic Affairs, The Ohio State University, 203 Bricker Hall, 190 North Oval Mall, Columbus OH 43210.

The Search Committee will begin screening dossiers on February 15, 1989 and will continue to receive nominations and applications until the Dean is selected.

The Ohio State University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply. (NW3269)A

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth

LECTURER IN ANATOMY AND HUMAN BIOLOGY
(Ref. A09/89

Applications are invited for a tenurable lectureship. The Department has strong teaching and research commitments in the Faculties of Science and Medicine. Extensive research, both basic and applied is being undertaken in such areas of human biology as (a) evolution, genetics, variation and ecology (especially primates); (b) comparative and functional anatomy, including biomechanics and morphometrics; (c) reproductive biology and (d) neurobiology. Techniques available include light and electron microscopy, surgical procedures, stereology, computer modelling, image and data analysis of various types. Applications are sought from individuals in the first two of the general areas outlined above and with any of the above techniques. Duties will include research development supervision and co-ordinatic courses, lecturing, labor and tutorial teaching. further details contact the of Department, Professor Oxnard, (09) 380 3298.

Salary range: A$30,73 A$40,100 p.a. Benefits inc superannuation, fares to Pert appointee and dependent fa removal allowance, study and long service leave. Condi of appointment will be specifi any offer of appointment w may be made as a result of advertisement.

Written applications qu reference number, telep number, date of birth, quali tions, and experience and names and addresses of thre erees should reach the Dire Personnel Services, The Un sity of Western Australia, lands 6009, Australia, by 3 M 1989.

Equal Employment Oppo ity is University Policy

(W5820)

SHELL RESEARCH LIMITED, SITTINGBOURNE, KENT

Microbial Physiologist

The Sittingbourne Research Centre of Shell Research Ltd has had a long term involvement in many aspects of Microbial Biotechnology. Current interests include investigations of the potential of micro-organisms to transform organic compounds either to high added value chemicals or to innocuous substances of low environmental impact. These studies of biocatalysis and biodegradation are being carried out by multidisciplinary teams which include chemists, biochemists, physiologists, molecular biologists and ecologists.

You will be a scientist with a degree in biochemistry, microbiology or a similar discipline. You should have a strong interest in the biodegradability of organic chemicals and in biodegradative mechanisms employed by micro-organisms, particularly mixed cultures. Some experience with model activated sludge or other biotreatment processes, with a knowledge of the performance of micro-organisms within mixed communities, would be beneficial.

The Research Centre is a modern, well equipped laboratory on a 500 acre rural site in Kent. We offer attractive salaries, good sports and social facilities and a staff restaurant.

If you are interested in the above position please write or telephone for an application form, quoting reference number SRC1/89 (Microbial Physiologist) to: Mrs Diane King, Personnel Division, Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG. Telephone: (0795) 412290. Closing date for applications is 16th February 1989.

Royal Dutch/Shell Group

CAREERS OF CONSEQUENCE

(8323)A

Scientific Computing Posts

The Daresbury Laboratory, operates a Synchrotron Radiation Source and a Nuclear Structure Facility, which are used by university groups from most parts of the country and abroad. In support of these facilities the Laboratory provides computing services.

Scientific Computing Ref DL/91

There are vacancies in the Synchrotron Radiation Computing Group for three Scientific Computing Posts.

The duties include maintenance and development of data acquisition programs that control experimental stations at the Synchrotron Radiation Source and support and development of data analysis packages for applications in the project areas of Soft X-Ray Spectroscopy, Non-Crystalline X-Ray Diffraction and Molecular Chemistry.

Experimental station control software run mainly on DEC LSI11/23 computers under RT-11. The data analysis codes run on DEC VAX computers under CMS, one with an attached FPS-264 array processor, or on the Laboratory's Convex C2 mainframe systems and working knowledge of Fortran and basic numerical analysis methods would be an advantage.

The successful candidates will join an existing team of scientific applications programmers but will be associated with one of the three projects and will be expected to liaise with in-house staff and external users. Active participation in the research programme would be encouraged.

For further details contact Dr E Pantos (0925) 603275.

Computational Scientist Ref DL/92

There is a vacancy in the Collaborative Computational Project for Protein Crystallography (CCP4).

The duties include maintenance and development of packages, which can be used by all scientists involved in protein structure determination by X-Ray crystallography. The packages run on DEC VAX computers under VMS, or on the Laboratory's mainframe CONVEX 220 computer under UNIX.

The person appointed will collaborate with protein crystallographers at Daresbury and from university groups in implementing new methods of data analysis and structure determination. Applicants should have experience in scientific data analysis. Solving computational problems and liaising with the protein crystallography community are important aspects of this work.

For further information contact Dr K Henrick (0925) 603528.

• Qualifications

Minimum qualifications required are:-
Higher Scientific Officer – Degree plus 5 years relevant experience or 1st or 2nd Class Honours degree plus 2 years post graduate experience.
Scientific Officer – Degree HNC/HND or equivalent.

• Pay

Appointments will be made at the Scientific Officer or Higher Scientific Officer grade depending on qualifications and experience. Salary is in the range £8,574 to £13,460 with further increments depending on performance.
The superannuation scheme is non-contributory.

For an application form please quote the relevant reference number and contact:
The Personnel Officer, Daresbury Laboratory, Warrington WA4 4AD.
Telephone (0925) 603467
(24 hour answering service).

CLOSING DATE:
Thursday 16th February 1989

(8321)A

Daresbury
SCIENCE & ENGINEERING RESEARCH COUNCIL

ASSISTANT PROFESSOR
MOLECULAR BIOLOGY OF RETROVIRUSES

The Division of Biology at Kansas State University has a tenure-track position available at the assistant professor level effective August 1989. Applications are invited from investigators with postdoctoral experience in the molecular biology of retroviruses and/or related oncogene expression. The division has a strong program in biologicl research, excellent facilities, and NCI Graduate Training Grant in Virology and Tumor Biology, and a distinguished faculty with several millions of dollars in extramural grants. The successful candidate will be expected to establish a vigorous and creative program in retrovirus and/or oncogene research, and to participate in graduate and undergraduate instruction. Candidates should apply by 15 February 1989 by submitting curriculum vitae, a brief description of future research plans, and having three letters of reference sent to:

Terry C. Johnson
Division of Biology, Ackert Hall
Kansas State University
Manhattan, KS 66505

KSU is an Equal Opportunity/Affirmative Action Employer

(NW3268)A

Research Fellow/ Staff Scientist

Applications are invited for a post-doctoral research fellow or staff scientist appointment in the Division of Geological and Planetary Sciences at Caltech. Applicants should have a Ph.D. in analytical chemistry, chemical physics or experimental physics with a sound background in laser spectroscopy and a strong interest in the development and application of laser ionization techniques. Research areas include development of laser resonance ionization for elemental and isotopic measurements in geological materials, isotopic and chemical analyses of primitive meteorites, kinetics of solid state diffusion in natural and synthetic silicates and oxides. Individuals with a particular interest in instrumental analysis and in the development of new and innovative research projects are encouraged to apply. Send CV and letter outlining research interests to **Prof. G.J. Wasserburg, Chairman, Division of Geological and Planetary Sciences, Mail Code 170-25, California Institute of Technology, Pasadena, CA 91125.** *Caltech is an equal opportunity/affirmative action employer.*

(NW3266)A

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INICAL INVESTIGATION MANAGER

e Medical Research Division of American Cyanamid Com- ny a diverse Pharmaceutical/ emical Company, has its Inter- ional Clinical Research Centre urope) at Richmond, Surrey.

To strengthen our clinical inves- ation group, we wish to appoint Clinical Investigation Manager o would have responsibility for management and co-ordina- n of the Company's projects in rope involving Phase I to Phase clinical research. A major re- irement of the job is the ability work with Research Physicians d Clinical Scientists in the des- and conduct of clinical studies d with the staff responsible for analysis of the research data.

It is expected that candidates l be educated to degree level d either have 2 to 4 years' exper- ce in clinical research with the armaceutical industry, or be rking in an academic environ- nt with some experience in the ical trials area.

The incumbent will be required travel abroad within Europe d to the USA. The salary and nditions of employment, inclu- g a company car will reflect the portance of the position within ernational Clinical Research.

To apply, please write with l CV to Carolyn Osbourne, Per- nel Officer, Cyanamid (UK), reham Road, Gosport, Hamp- re, PO13 0AS, or ring our ansa- one on Fareham (0329) 230941 an application form any day er 12 noon. (8289)A

DIRECTOR

British Geological Survey, Keyworth, Nottingham
Salary up to £33,800

The Natural Environment Research Council is seeking a Director for the British Geological Survey to succeed Mr Geoffrey Larminie, OBE, upon his retirement in August 1989. The Director will be responsible to the Director of Earth Sciences, NERC, for the scientific leadership and management of BGS which has a staff of about 750 at its headquarters in Keyworth (Nottingham), Edinburgh and four small regional offices. The annual budget of BGS is about £24m.

Applicants should have an international reputation in the Earth Sciences and previous experience in the management of geological personnel and projects. Experience of working with both industry and government will be an advantage.

Starting pay on appointment will be on the scale £32,219 to £33,819, with a further increment to £35,457 depending on performance. A non-contributory pension scheme is available, plus a generous annual leave allowance. Some assistance towards relocation costs may be available.

Application forms and further information may be obtained from Lorraine Nowlan at the address given below or by telephoning (0793) 411500 or (0793) 411531. Completed applications, including a full CV should be sent to Lorraine Nowlan, NERC, Polaris House, North Star Avenue, Swindon, Wiltshire SN2 1EU, by 28 February 1989.

The Natural Environment Research Council is an Equal Opportunities Employer.

Natural Environment Research Council

(8311)A

UPPSALA UNIVERSITY

Applications are invited for a tenured

PROFESSORSHIP IN ZOOPHYSIOLOGY

at the University of Uppsala, Sweden (starting salary: from 210 000 SEK).

Subject area for the chair:
"The position is in the field of animal physiology and integrated functions in a broad sense such as the physiology of organs and organisms and cell physiology aiming at analytical studies of physiological mechanisms and adaptation."

The application should be directed to the Swedish Government and should be received at the Registrar's Office, Uppsala University, Box 256, S-751 05 Uppsala, Sweden, *no later than February 22, 1989*.

The applicant should submit a curriculum vitae (duly signed and witnessed), a short written account of his/her scientific and pedagogic activities, certificates and such other documents as to which the applicant wishes to refer, a list of scientific publications and the scientific publications referred to numbered and sorted according to the list of publications. All in four copies.

For further information, please contact the Dean of the Division of Biology, telephone 00946-18-17 45 26.

(W5837)A

PROTEIN CHEMIST

We have one or more openings for post-doctoral protein chemists to explore some new ideas for the site-specific modification of antibodies for diagnosis and therapy, particularly of cancer. The experimental programme is well funded from Industrial and charitable sources and is expected to give considerable scope for novel, useful research. The department is equiped with all necessary apparatus (in particular, a major mass-spectrometric facility dedicated to protein work, an ABI sequencer, and an ABI peptide synthesizer). We have excellent clinical and industrial contacts. Salary approx. 50'000–60'000.— Swiss Francs. The appointment is for two years in the first instance: one post is available immediately.

Please send C.V. and representative reprints or preprints to:

Prof. R. E. Offord,
Department of Medical Biochemistry,
University Medical Centre,
1 rue Michel-Servet,
1211 Geneva 4, Switzerland,
Tel. 22'90'15. (W5832)A

Sigma Tau

a leading Italian Pharmaceutical Company is establishing a new Cardiovascular Research Centre in the Milan area

The following positions are vacant:

Head of team in vivo Pharmacology

This is a position for an experienced and innovative pharmacologist, head of a team of approximately ten, able to coordinate with other multidisciplinary teams. The primary focus of this team is on animal models of genetic cardiovascular diseases for which a long lasting experience already exists within the people of the centre. Pathophysiological alterations and the effect of new drugs will be studied on these animals in collaborations with other teams dealing with medicinal chemistry, genetics, protein chemistry, molecular biology, cell pharmacology and clinical studies in man with similar diseases.

Molecular Biologist

This is a position for a candidate having at least 4-5 years of experience in a relevant area of molecular biology, head of a small team, able to coordinate with the other teams. The primary focus of this team is on the identification and characterization of the genes causing the cardiovascular diseases of the animal models studies within the centre. The possibility to study humans with similar diseases is also encouraged.

Senior Research Chemist

This is a position for a candidate having a proven wide experience in organic synthesis and computerized medicinal chemistry able to take the responsibility of a small group of chemists working in collaboration with other chemical research groups.

A highly competitive salary is offered for these three positions. Fringe benefits will also be commensurate with experience and qualifications. Relocation assistance is available when appropriate.

Please send a detailed curriculum vitae to:

Dr. R. Colasante
SIGMA TAU FINANZIARIA
Via Sudafrica, 20
00144 ROMA
ITALY

(W5809)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE
INSTITUTE OF MEDICAL GENETICS
(DEPARTMENT OF MEDICINE)

POSTDOCTORAL RESEARCH OFFICER

A three-year post, funded by the Wellcome Foundation, is available immediately to join a research team working on the molecular genetics of X-linked hypohidrotic ectodermal dysplasia. The work will involve further molecular characterisation of the disease-related mutations. Practical experience of current molecular biological techniques would be an advantage, but is not essential.

Salary within the scale for University Research and Analogous Staff Grade 1A, starting point £9,865 per annum.

Applications in the form of a curriculum vitae (quoting ref. No. M6/218) with names and addresses of two or more referees, should be sent to the **Registrar and Secretary (Personnel Office), University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN (Tel. No. 755944 ext. 2296)** from whom further particulars are available.

Informal enquiries can be made to Dr N.S.T. Thomas (Tel. No. 0222-7622901).

Closing date for applications: 3 weeks from the appearance of this advertisement. (8312)A

NAGOYA UNIVERSITY

Research Institute for Biochemical Regulation, School of Agriculture

Visiting Professor

(1 year, senior or junior scientist)

and

Postdoctoral Fellowships

(1-2 year, citizen of US, UK, France and W-Germany)

for research in plant or animal cell biology, molecular biology, developmental biology, and biochemistry, etc. For details contact and send CV and names of scientists willing to supply letters of reference to: **Director, Dr. T. Akazawa, Research Institute for Biochemical Regulation, School of Agriculture, Nagoya University, Chikusa, Nagoya 464-01, Japan.**

FAX 052-781-4447. TELEX 4422120 ANUNAG J.

(W5831)A

FOODS AND NUTRITION

12-month tenure-track position; Department of Foods and Nutrition, University of Georgia. Starting Date: Sept. 1, 1989. Position title: Assistant Professor of Foods and Nutrition. Salary dependent on qualifications. Position includes teaching (graduate and undergraduate), research, and advising students. Applicants must have earned doctorate. Faculty are expected to conduct research and seek extramural support for the same. Send copies of vita, transcripts and four letters of recommendation to: **Dr. Roy J. Martin, Professor and Head, Department of Foods and Nutrition, College of Home Economics, The University of Georgia, Athens, Georgia 30602.** Phone: 404-542-4875. Deadline: March 31, 1989. *The University of Georgia is an EEO/AA Employer.*

(NW3270)A

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nature

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SENIOR BIOCHEMISTS
RESEARCH BIOCHEMISTS
RESEARCH CHEMISTS & ANALYTICAL ORGANIC CHEMISTS
SENIOR MECHANICAL DESIGN ENGINEER
ENGINEERING MANAGER
PROJECT LEADER
SYSTEMS ANALYST & SENIOR PROGRAMMERS
CHROMATOGRAPHY/ELECTROPHORESIS ENGINEERS

We are a UK Biotechnology start-up company with the aim of becoming the leading worldwide provider of advanced products and services for carbohydrate analysis. Our products are based on powerful and proprietary technology from the University of Oxford. We have immediate openings in the Instrument Systems Group, Fine Chemicals Group and Research Services Group.

Your enthusiasm, professionalism, superior technical and research skills and flexibility to work in a multi-disciplinary team on an aggressive schedule will make you a successful candidate.

If you would like to play a key role in a fast growing, innovative and friendly organisation, apply now. We are searching for top percentile professionals and offer highly competitive renumeration and benefits package, including equity participation.

SENIOR BIOCHEMISTS

Oxford GlycoSystems specialises in the manufacture of carbohydrate related fine chemicals and provides a unique and exceptional set of research services. The company places great emphasis on the quality of its scientists and is currently seeking:

Senior Biochemists of exceptional calibre for supervisory positions in the Research and Research Services groups. The position in the Research group will involve responsibilty for developing new products for the Fine Chemicals business, while the position in the Research Services group involves responsibility for the entire spectrum of company business, from customer contact to actual analysis of samples. For both positions, a proven record in analytical biochemistry and significant managerial experience are required. A knowledge of techniques for glycoprotein and/or carbohydrate analysis is preferred, but not essential. These positions represent a unique opportunity for senior biochemists to create and manage their own group of scientists, while also being active at the bench, and free from the routine tasks of maintaining and administering a laboratory.

RESEARCH ASSISTANTS

Research Assistants will report directly to Group team leaders in the Research and Research Services. Candidates should have an honours degree (or equivalent), and considerable experience in protein chemistry. An above average salary and benefits package are available.

RESEARCH BIOCHEMISTS

The Research Biochemist will join a young and dynamic group of scientists engaged in research on glycoprotein structure and function. The successful candidate will soon head their own sub-group. An honours degree and Ph D, or equivalent experience, are required.

BIOMEDICAL INSTRUMENTATION ENGINEERING

Applications are invited to join OGS as the **Manager** of Engineering of the Instruments Systems Group. This newly formed company specialises in Bioanalytical instruments used in the Biotechnology Research industry and is producing the world's first carbohydrate sequencer.

Experience as a manager of product development in the area of scientific or medical instruments is absolutely required. Experience in managing five of the following disciplines of engineering is required:
software: machine control and user interface;
detection systems: light or other radiation;
analogue electronics: small signal simplification and conditioning;
digital electronics: I/O, CPU board design;
hydraulics: small tubes and valves carrying chemicals (eg, HPLC);
mechanical apparatus: small scale mechanisms.

Additionally, the successful candidate will have had experience managing the entire product development life cycle from conception to volume shipments of scientific instruments. He/she must have five years experience managing a multi-disciplinary group of ten or more professional engineers and budget responsibility experience in excess of £1 million per year. A talent for working effectively with marketing will be highly regarded.

OGS is searching for a top percentile individual with excellent communication skills and an ability to build up a dedicated team of product champions.

We are also looking for technical professionals with product development experience in the following fields: microprocessor controlled analytical and laboratory instruments, real time process systems, analog design and development, software, optics, physics, miniature fluid handling, mechanisms and mechanical packaging. If you have mechanical engineering experience, send us your c.v.

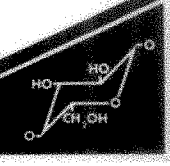
If you fit any of the job descriptions listed please let us know. If you know someone who fits any of these job descriptions, let them or us know. If you would like to work for any of these people once we find them, let us know that too.

Apply enclosing a full c.v. to:

Oxford GlycoSystems

Professional Staffing, Oxford GlycoSystems Limited,
Unit 4, Hitching Court, Blacklands Way,
Abingdon Business Park, Abingdon, Oxon,
OX14 1RG.
Tel: 0235–553066.

(8324)A



POSTDOCTORAL RESEARCH POSITION

Collaborative Research Programme: Dyson Perrins Laboratory, Oxford University in association with Celltech Limited

This postdoctoral research position, based at the Dyson Perrins Laboratory, Oxford, represents an exciting opportunity to collaborate with Celltech – Europe's foremost independent biotechnology company. The research involves the synthesis of prodrugs designed as part of a novel approach to cancer chemotherapy. Experience in synthetic organic chemistry would be an advantage. The post is to start as soon as possible and will be funded until April 1991. Salary will be up to £15,095 pa; starting point depending on age and qualifications.

Applicants should send a curriculum vitae to Dr A J Pratt, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY and should ask two referees to write directly on their behalf to Dr Pratt.

CELLTECH

(W5839)A

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nature

The international journal of science with 500,000 readers throughout the world seeks

A TALENTED BIOLOGIST

Essential qualifications:

- A sound education in some field of biology.
- A critical and lively interest in science in general.
- A sense of literacy.
- Reseach experience.

Applications are invited from people seeking a career in the communication of science. The successful applicant will join the team responsible for assessing and editing biology manuscripts, initially (at least) in London. There will be opportunities for contributing to *Nature* in other ways. Research experience in neurobiology would be particularly welcome, but those with other interests should not be deterred from applying.

To apply, please send your *curriculum vitae* to the Editor, with a detailed account of your education, research experience and literacy skills, at 4 Little Essex Street, London WC2R 3LF, or 1137 National Press Building, Washington, DC 20045, as soon as possible and before 24 February. (8317)A

Research Associate

to conduct research as follows:

determine the roll of the cell and mutation in the hemagglutinin (HA) gene in determining host range; investigate the role of glycosylation of he HA in determining how well new clinical isolates replicate in cell cultures; investigate feasibility of cloning HA genes directly from clinical sample to determine if the number of oligosaccharides on the HA or the position at which the HA is glycosylated is changed when the virus is grown in cell culture. Salary: $20,900 per year/40 hour week. Requirements: Ph.D., or equivalent, in cellular or molecular biology. Applicant must have at least two publications in nucleic acid (DNA or RNA) protein interaction research. Applications to: **Consuelo Walker, ALC Specialist, Job Service, 505 Washington, St. Louis, Missouri 63101**. Please refer to Job Order Number 237719. (NW3274)A

The David Mahoney Institute of Neurological Sciences (INS) of the University of Pennsylvania is recruiting a

Developmental Neurobiologist

at the rank of Assistant or Associate Professor. We are particularly interested in people working at the molecular or cellular level, either in genetics or in other aspects of nervous system development. The INS has over 100 members and already has several excellent laboratories working on cellular, molecular and biochemical aspects of neural development and regeneration. Applicants should send their curricula vitae and the names and addresses of three references to: **Michael E. Selzer, MD/PhD Department of Neurology Hospital of the U or PA Philadelphia, PA 19104-4283.** ***University of Pennsylvania is an Equal Opportunity/Affirmative Action***

(NW3264)A

UNIVERSITY OF MANCHESTER

POSTDOCTORAL POSITIONS: EPITHELIAL CELL PHYSIOLOGY

Applications are invited for two Research Associates in the Department of Physiological Sciences to study stimulus-secretion coupling, regulation of intracellular pH and Ca and anion transport in exocrine gland cells. One position will be primarily concerned with patch-clamp recording, the other with measuring intracellular ion concentrations by single-cell fluorescence microscopy. Persons appointed will join a research group, headed by Professor Maynard Case, investigating a number of aspects of epithelial transport and secretion. Applicants should have a Ph.D. in a relevant subject. Some experience with electrophysiology or isolated single cells an advantage. Appointments funded for three years. Salary range £9,865-£11,680 p.a. depending on age and experience. Applications, with c.v. and the names and addresses of two scientific referees, to **Professor R.M. Case, Department of Physiological Sciences, the University, Manchester M13 9PT. Phone: 061 275 5463 Fax: 061 275 5572.** Informal telephone enquiries welcome. *The University is an equal opportunities employer.*

(8307)A

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

SCHOOL OF EARTH SCIENCI

HYDROLOGIST – SOIL PHYSICIST

Applications are invited appointment as lecturer in Physics in the School of E Sciences, available for a perio three years. The Lectureshi equally supported by Flin University and by the C.S.I.R Division of Water Resources.

The School of Earth Scienc a partner in a Consortium wi operates a Centre for Rese in Groundwater Processes sp sored by the Australian W Research Advisory Council. consortium comprises C.S.I.R.O. Divisions of W Resources and of Soils, toge with the State Departments Engineering and Water Sup and of Mines and Energy, in a tion to Flinders University, the facilities and resources of th institutions are avaialable to Centre.

The Centre is active in a rang research programs focussing u the unsaturated zone, which clude: land and water salinisati evapotranspiration, microbiol cal processes, contaminant hyd ogy, aquifer processes and hyc chemistry, inter-annual clim variability and Australian drou cycles, palaeo-climate studies.

The successful applicant will required to service course w teaching within the Univers assist in the supervision of growing school of Postgradu Students and play an active r in the research program of Centre. Further information n be obtained from the Dean of School of Earth Sciences, t phone (08) 275 3911, fax (08) 2676.

Salary Scale: A$30,737 A$40,100 p.a. (An appointm will not be made above the si level of the scale, viz. A$37,424

Written applications giving details of qualifications and exp ience, and the names and addr ses of three referees of whom c fidential enquiries may be ma should be forwarded, in duplica to the Director of Adminstrati and Registrar, The Flind University of South Austral Bedford Park, S.A., 5042, by March 1989.

Equal Employment Opportu ity is University Policy

(W5819)A

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nature

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Opportunities in Drug Discovery Research in Japan

Pfizer Central Research at Nagoya, Japan, is offering excellent career opportunities in our expanding drug discovery research programs. The laboratories located 35 km south of Nagoya city are an integral part of Pfizer Central Research worldwide and Pfizer Taito Co. Ltd., a Pfizer Inc. subsidiary. We are seeking Japanese scientists at the Ph.D. level with degrees in Biochemistry, Immunology, Pharmacology, Microbiology, and Organic Chemistry, and 1-3 years of post-doctoral or industrial experience. In addition to strong technical expertise, all positions require extensive English communication skills and computer literacy. Specific requirements are as follows:

Immunologist/Biochemist

The successful candidate will have work expertise in cytokines, cell surface receptors, and lymphocyte activation. Initially, he or she will be responsible for developing *in vitro* assays aimed at accomplishing several goals, including evaluation of the role of different mediators in the immune response. Knowledge of tissue culture techniques is essential.

Pharmacologist

A broad knowledge of, and experience with, various animal models of inflammation is essential. The successful candidate will be responsible for developing animal models of arthritis and autoimmunity. Some background in general pharmacology and cellular immunology is desirable.

Microbiologist

A strong background in biochemistry and molecular biology of micro organisms is essential. Knowledge of taxonomy is desirable.

Natural Products Chemist

The successful candidate will become a member of our discovery fermentation group. Experience in modern isolation techniques and spectral methods is essential.

Organic Chemist

Applicants should have extensive expertise in organic synthesis. Responsibilities include design and synthesis of biologically active compounds. Keen interest in the biological aspects of drug discovery is essential.

Pfizer Taito offers a competitive salary and excellent benefits, including a relocation package. Qualified applicants should submit their resumes and a brief description of their research accomplishments to **Mr. Kenji Takamatsu, Director of Personnel, Pfizer Taito Co. Ltd, P.O. Box 226, 1-1 Nishi-Shinjuku 2-Chome, Shinjuku, Tokyo 163, Japan.** (W5784)A

Pfizer 台糖ファイザー株式会社

ファイザー名古屋中央研究所

UCLA

CELL AND MOLECULAR PHYSIOLOGY

The Department of Physiology invites applications for tenure track faculty positions at the level of

Assistant Professor

Outstanding candidates working in any area of contemporary physiology will be considered, but those in smooth muscle, reproduction and development, endocrinology, sensory transduction, and the cardiovascular system are encouraged to apply. Successful candidates are expected to have a Ph.D. or M.D. with post-doctoral experience, to provide evidence that they can pursue a vigorous, independent research program and to participate in our teaching program.

Resources and an excellent environment exists for developing an academic career within an interactive community. Interested scientists should send their curriculum vitae, a statement of present and future research interest, and names of three references to: **Ernest M. Wright, D.Sc. Professor and Chairman, Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90024-1751.**

The University of California is an Equal Opportunity Affirmative Action Employer. (NW3265)A

EXECUTIVE DIRECTOR
QUATERNARY SCIENCES CENTER, DESERT RESEARCH INSTITUTE
UNIVERSITY OF NEVADA SYSTEM

The Quaternary Sciences Center of the Desert Research Institute is seeking an Executive Director of national reputation to provide administrative and scholarly leadership for its expanding basic and applied research programs in Anthropology, Archaeology, History, Paleobotany, Paleozoology and Quaternary Geology. The Center has an agenda to expand its research into improving understanding of global environmental change in arid environments. The successful applicant must have demonstrated the administrative and interpersonal skills required to foster the productivity and creativity of a small group of research scientists and to be an effective advocate for the Center. Applicants should have significant research achievements in the Quaternary sciences. Experience in archaeology is desired and a Ph.D. is preferred. Salary is state-funded in the $75,000 to $80,000 range negotiable commensurate with applicant's qualifications and experience.

The Desert Research Institute is the statewide research division of the University of Nevada System, Research is managed in five centers: Atmospheric Sciences, Biological Sciences, Energy and Environment Engineering, Water Resources and Quaternary Sciences. DRI's annual budget now exceeds 16 million and is growing at 15% annually. The Quaternary Sciences Center (formerly the Social Sciences Center) has an annual budget of over a million dollars in grants and contracts and a full-time staff of 21 professionals and technicians. Center research is concentrated on arid and semi-arid lands of the world with particular emphasis on the Intermountain West; opportunities exist for developing additional programs in Quaternary studies. Applications should include a résumé and statement of interest. Initial screening of applications will begin by May 1, 1989. Placement is desired by July 1, 1989 although a later date is negotiable. Applications should be addressed to **Dr. Peter Wigand, Chairman, Quaternary Sciences Center Executive Director Search Committee, Desert Research Institute, University of Nevada System, P.O. Box 60220, Reno, NV. 89506.** For further information call Dr. Peter Wigand at (702) 673–7387. *The Desert Research Institute is an affirmative action equal opportunity employer.* (NW3275)A

Director

Parke-Davis Research Institute
Sheridan Park Research Center, Mississauga, Ontario, Canada

The Parke-Davis Research Institute is dedicated to toxicological research and safety testing of new pharmaceuticals as part of the worldwide drug development efforts of the Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company.

We are seeking a strong individual with a DVM, MD or Ph.D. in Toxicology or equivalent advanced degree in science. Certification in Pathology, Toxicology or equivalent is desired.

This senior research management position is directly responsible for the operation, planning and conduct of toxicology studies by a large group of scientists. A minimum of 8-10 years of research experience in toxicology/pathology of laboratory animals from safety evaluations is desired, preferably with experience in a multi-disciplinary environment of toxicology research. Working knowledge of all aspects of toxicity testing, including general toxicology, pathology, mutagenesis, clinical laboratory sciences and reproductive toxicology will be necessary, together with knowledge of Good Laboratory Practice regulations, worldwide testing and regulatory guidelines and experience in regulatory affairs.

This leadership position requires an exceptional person with long-term growth potential, excellent publication record, national/international recognition and demonstrated record of independent biochemical, toxicological or biological research.

Excellent written and oral communications skills and interpersonal effectiveness with management background are essential. This senior scientist would also have experience in successfully leading scientists, encouraging open communications and working in a complex matrix organization, actively interacting with senior research management, and having administrative control of a large budget and scientific projects.

This high quality laboratory constitutes a part of the worldwide toxicology research efforts and the position reports to the Vice President, Pathology and Experimental Toxicology, located at the division research headquarters in Ann Arbor, Michigan, USA.

Enjoy an excellent program of benefits, relocation and compensation, in addition to a high quality of life environment, which makes this position very attractive for a long-term career with Warner-Lambert.

In compliance with Canadian Government Regulation, preference will be directed to Canadian citizens or permanent residents.

Join this respected worldwide leader in pharmaceutical research. Send curriculum vitae and references in confidence to: Mr. T. Romps, Human Resources Representative, PARKE-DAVIS Pharmaceutical Research Division, 2800 Plymouth Road, Ann Arbor, MI 48105, USA.

PARKE-DAVIS

DIVISION OF WARNER LAMBERT COMPANY

"Equal Opportunity in Action"

(NW3271)A

UNIVERSITY OF HONG KONG

Senior Lecturer/ Lecturer in Zoology

Applications are invited for Senior Lectureship/Lectureship the Department of Zoolo Preference will be given to app cants specializing in any area experimental zoology, develo mental biology or fish biology. T appointee will be expected share in the teaching of the gene area of zoology as well as devel ping courses in his/her speciality

Annual salaries (superann able) are on the scales: Seni Lecturer: HK$311,400–418,380 points) (approx. £22,400–30,10 Lecturer: HK$200,340–334,8 (11 points) (approx. £14,41 24,090). (Sterling equivalent as January 13, 1989). Starting sala will depend on qualifications a experience. At current rates, sal ries tax will not exceed 15½% gross income. Children's educ tion allowances, leave, and mec cal benefits are provided; housi or tenancy allowances are al provided in most cases at a char of 7½% of salary.

Further particulars and applic tion forms may be obtained fro Appointments (35968), Associ tion of Commonwealth Univer ties, 36 Gordon Square, Lond WC1H 0PF, UK, or from t Appointments Unit, Registr University of Hong Kong, Ho Kong. Applicants are additional requested to provide, in respect their major research area: a she descriptive title; and a bri summary of the work they ha undertaken and the direction which their work is proceedin Closes *31 March 1989*.

(W5833)A

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nature

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CONSULTING

U.S. scientific consulting firm offering assistance in analysis of data and communication of findings in written reports and public presentations to a variety of forums seeks Ph.D.'s in biological and biochemical sciences and M.D.'s interested in worldwide consulting opportunities. Disciplines of interest include epidemiology, biostatistics, toxicology/pharmacology, cancer research, pathology, immunology, allergy, etc., and any of the medical specialties. Applicants will provide assistance on various projects. The position is intended for those people looking to supplement ongoing activities. Travel can be minimized. Retired individuals are welcome as are all academic professionals.

Please reply with résumé and rate structure to: **Chief Executive Officer, Suite 206, 3421 M Street, N.W., Washington, DC, USA 20007.**

(NW3272)A

ASSISTANT ENTOMOLOGIST

Full-time, tenure-track, 100% research. Develop research program on the management and/or eradication of tephritid fruit fly pests. Minimum Qualifications: Ph.D. degree (by closing date) in entomology or related field from an accredited university. Knowledge and experience with IPM, proper pesticide use, statistical methods. Demonstrated communication skills. Position located on Kauai Island. Available 1 May 1989. CV, reprints, official transcripts & three letters of references to **Dr. Ronald F.L. Mau, Entomology Department, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822** by March 15, 1989. Details (808) 948-7063; Fax # (808) 948-6442. EOE/AA

(NW3273)A

UNIVERSITY OF EDINBURGH

Department of Forestry and Natural Resources

Post-Doctoral Research Fellowship

PLANT PHYSIOLOGIST

Applications are invited for an 18-month post-doctoral position, with possible extension to 3 years, based at the Edinburgh Research Station of the Institute of Terrestrial Ecology. The successful applicant will work closely with tropical tree improvement projects led by Dr Roger Leakey and in collaboration with tree physiologists at the University of Edinburgh. The position, funded by Shell Research Ltd., is for investigations into the sources of variation in rooting ability in tropical Eucalypts and, subsequently, for the development of techniques to propagate difficult-to-root clones. Experience in environmental physiology is desirable.

Salary range £9,305-14,825 (min. age 27 £11,015) depending on age and experience.

Further particulars may be obtained from the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS.** Applications should also be sent to this address together with names of two academic referees to arrive no later than 23rd February, 1989. Please quote reference No. 5632. (8298)E

POSTDOCTORAL FELLOWSHIP

available now to study repair of 06-methylguanine in DNA and human tumor suppressor genes in the laboratory/clinical collaborative program of the Molecular Genetics and Carcinogenesis Laboratory of The Cross Cancer Institute. Two years with third possible. Background in DNA repair and molecular techniques advantageous. $23,815–$30,449.

Send curriculum vitae and names of three references to **Dr. Rufus S. Day, III, Cross Cancer Institute, 11560 University Ave., Edmonton, Alberta, T6G 122, Canada.** (NW3229)E

nature

Still first with the best in science

LINCOLN COLLEGE
University College of Agriculture
New Zealand

POSTDOCTORAL FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship in the laboratory of Professor D. W. Bullock to work on *trans*-acting factors involved in the regulation of gene expression by progesterone. Applicants should have a Ph.D or equivalent in biochemistry or molecular biology with experience in DNA cloning and sequencing. The post is funded by a Medical Research Council grant for three years at an initial salary of NZ$35,000. per annum.

Lincoln College has made a major commitment to molecular biology and the appointee will join a new and expanding research group with strong interests in gene transfer and transgenic animals.

Applications should include a curriculum vitae, list of publications and names and addresses of three referees.

Conditions of Appointment may be obtained from the undersigned, with whom applications close on 13 February 1989.

G. A. Hay,
Registrar,
Lincoln College,
Canterbury,
New Zealand.
(W5816)E

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
Department of Physiology and Pharmacology

POSTDOCTORAL FELLOWSHIP

Applications are invited for a Wellcome Trust Postdoctoral Fellowship to investigate the regional haemodynamic effects of central neuropeptides in hypertension. The position is available for two years in the first instance, and would be best suited to a person with experience of the appropriate techniques.

Applicants should send their curriculum vitae and the names and addresses of 2 referees to: **Dr S M Gardiner, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, Tel: 0602 421421 Ext 3344.**

Closing date 28th February 1989. (8273)E

UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY

(1) RESEARCH ASSOCIATES

TWO post-doctoral fellows are required to join a team which has recently isolated cDNAs encoding the complete sequence of the cytoskeletal proteins vinculin and alpha-actinin (Biochem.J 245(1987)595; J.Biol. Chem. 262(1987)17623; Cell 52(1988)159). Interest in these proteins stems from the observation that they are associated with actin in cell-cell and cell-matrix adherens junctions, structures which are disrupted in tumour cells.

Projects will include the use of (1) site-directed mutagenesis to define binding domains in these proteins (2) expression systems for the purification of protein fragments for crystallization studies (3) genomic cloning to establish the origin of protein isoforms.

(2) ONE YEAR POST-DOCTORAL TRAINING FELLOWSHIP

The post, which is associated with the above projects, is ideally suited for someone wishing to gain experience in recombinant DNA technology.

Applicants should have a Ph.D. in Biochemistry or related discipline. Experience in DNA technology is desirable though not essential, as training is available for highly motivated candidates of proven ability. The posts are available for a maximum of three years. Starting date not later than April 1st 1989.

Salary according to age and experience, on scale IA: £9,865 to £15,720.

Applications including a detailed c.v. and the names of two referees to Dr. D.R. Critchley, Department of Biochemistry, University of Leiceter, Leicester LE1 7RH (Tel. no. 0533 523477). (8264)E

THE ROYAL VETERINARY COLLEGE
University of London
Department of Veterinary Pathology

POSTDOCTORAL RESEARCH FELLOWSHIP

A three year post is offered to develop an anti-idiotype vaccine against porcine paravovirus to control reproductive losses. Experience with monoclonal antibody production and some molecular biology would be an advantage.

The appointment will be for a term of three years, subject to a satisfactory annual report.

Interested persons are encouraged to get in touch with Dr N Edington.

SALARY SCALE, University Research Grade 1A, ranging from £11,515 to £17,370 per annum. Starting salary according to age and experience.

Application form from the **Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU (tel no: 01-387 2898).** (8288)E

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS

in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from **Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G.** (W5706)E

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1989 AWARDS

Next deadline: 10 February 1989

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are usually awarded initially for one year, but with the possibility of renewal for a second year. In cases of exceptional scientific merit, however, the selection committee can award a 2-year fellowship. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 10 February 1989. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 17 April 1989.

Application forms and further details may be obtained from **Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.** Inquiries should be accompanied by a self-addressed adhesive label. (W5559)E

UNIVERSITY OF BIRMINGHAM
SCHOOL OF BIOCHEMISTRY

Postdoctoral Research Fellowship (Ref BC1975)

Graduate Research Associateship (Ref BC1976)

Applications are invited for the above positions to work on the genetic basis of reduced sulphur-oxidation. The aim of the project, which is funded for three years by an MRC grant to Dr R H Waring, is the characterisation of the enzyme system controlling S-oxidation in man, and will involve protein purification and the construction and screening of cDNA libraries. This will be linked with clinical studies at the Queen Elizabeth Hospital, Birmingham on correlations with restriction fragment length polymorphisms and chronic liver disease. The project will start on or after 1 April 1989. Candidates for the Research Fellowship should have experience in the techniques of molecular biology. Candidates for the Research Associateship should hold a good honours degree in the Life Sciences and have an interest or experience in protein purification. Registration for a higher degree may be possible.

For further particulars of the above posts and an application form, telephone 021–414 6383 quoting reference number BC1975 or BC1976. Applications (3 copies) should be sent to the **Senior Assistant Registrar, Faculty of Science, PO Box 363, Birmingham B15 2TT** by 20 February 1989.

AN EQUAL OPPORTUNITIES EMPLOYER. (8271)E

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
MOLECULAR BIOLOGIST
Post-doctoral Fellowship/Pre-doctoral Research Assistantship

Available from January 1989 to work on a 2 year MRC funded project to find new ways to express AIDS virus antigen, in eukaryotic cells. Experience in molecular biology techniques would be an advantage. The successful candidate will join an active research team housed in a very well equipped suite of laboratories in the Department of Medical Microbiology.

Informal enquiries can be made by telephoning Dr A Coates on 01-377 7259.

Applications including CV and the names of two referees should be sent to:

Dr A Coates
Dept of Medical Microbiology
The London Hospital Medical College
Turner Street London E1 2AD

within 2 weeks of the date of this advertisement. (8270)E

AFRC Post-Doctoral Research Fellow

Salary Scale: £10,071 to £13,779

Applications are invited for a Post-Doctoral Research Fellowship, funded by the Agriculture and Food Research Council for a period of 3 years, to investigate mechanisms of signal transduction between perception of light and induction of flowering in higher plants. The work will be supervised jointly by Dr. P. Lumsden (Lancashire Polytechnic) and Dr. A. Hetherington (Lancaster University).

Application forms and further particulars are obtainable, quoting reference number AA/555, from Personnel Services, Lancashire Polytechnic, Preston PR1 2TQ. Tel: (0772) 262027 (24 hour answerphone)

Closing Date:
17th February, 1989.

The Polytechnic is working towards equality of opportunity for all.

Lancashire Polytechnic

(8322)E

THE UNIVERSITY OF AUCKLAND

New Zealand

POSTDOCTORAL FELLOWSHIP

Applications are invited for a University Grants Committee Postdoctoral Fellowship to commence during the period 1 February 1989 to 30 June 1990.

Department of Biochemistry (Dr H. M. Nonhebel — supervisor). "Biosynthetic Control of Levels of Plant Hormone Indole-3-Acetic Acid". Applicants should have experience in one or more of plant tissue culture — enzyme extraction from plant tissue — extraction, analysis and *in vitro* translation of m RNA from plant tissues.

The Fellowship is available for two years, and emolument is a stipend of NZ$35,000 per annu Applicants should have complet all requirements for the award PhD before taking up a Fello ship. One return airfare is p able.

Conditions of Appointment a Application Forms are availal from Appointments (3598 Association of Commonwea Universities, 36 Gordon Squa London WC1H 0PF, or from t Assistant Registrar, Acaden Appointments, University Auckland, Private Bag, Au land, New Zealand. Applicatic should be submitted as soon possible, and preferably by *March 1989.*

The University of Auckland
An Equal Employment Opportunity Employer
(W5836)E

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ANTI-CANCER FOUNDATION OF THE UNIVERSITIES OF SOUTH AUSTRALIA HANSON FELLOWSHIP IN CANCER RESEARCH

Applications are invited from males and females for a Fellowship in Cancer Research. The occupant can undertake studies in any clinical or basic field of cancer research, although the Foundation would give some preference to a suitable person working in the area of Radiation Oncology.

The successful applicant will work in South Australia in either a Hospital Research Laboratory or University Department, and may take up the Fellowship as soon as convenient after 1 June 1989.

ELIGIBILITY: The Hanson Fellowship is intended to support persons below age 35 who are well established in a research career, as assessed by published works and reports of referees.

HOW TO APPLY: Applicants should first correspond with the head of a department in one of the relevant institutions in South Australia and construct a research project which is acceptable to that department and to the applicant's own interests and previous experience.

Applications should be submitted directly to the Foundation, and should include two copies of a detailed research project and of the candidate's curriculum vitae, which should include a list of published work, grants received and outline of previous research experience. The names of three referees should be included.

SELECTION: Selection will be based on an assessment of the applicant's record and the scientific worth of the research project. An interview will be conducted with the applicant (or nominee if the applicant resides outside Australia) following satisfactory reports by outside assessors.

SALARY AND CONDITIONS: The salary being offered will commence between AUS$28,118 and AUS$40,100 depending on past experience and qualifications and will thereafter follow the appropriate Research Salary Scales of the Universities of South Australia. The three year period of appointment is not renewable.

Maintenance Grant: up to AUS$3,000 per year. The institution where the work is carried out will determine all other conditions of employment.

Applications should be addressed to: The Chief Executive Officer, Anti-Cancer Foundation, PO Box 160, North Adelaide, SA 5006, telephone (08) 267 5222.

Closing date for applications: 24 March 1989. (W5808)E

PUBLIC HEALTH SERVICE

ALCOHOL, DRUG ABUSE AND MENTAL HEALTH ADMINISTRATION
NATIONAL INSTITUTE OF MENTAL HEALTH

FELLOWSHIPS IN BASIC AND CLINICAL NEUROSCIENCE

An extensive basic science and clinical research component of the NIMH Intramural Research Programs is located at the NIMH NEUROSCIENCES CENTER AT SAINT ELIZABETH'S in Washington, D.C. under the leadership of Drs. Rex Cowdry, Carl Merrill, Daniel Weinberger, and Richard Wyatt. This program conducts a broad range of neuroscientific investigations, including studies in: molecular neurobiology; neurochemistry of amines; peptides and receptors; CNS mitochondrial studies; identification of disease-specific proteins; neuropathology; neuropsychopharmacology; CNS tissue transplantation; neurovirolgy-immunology (e.g., studies of neurotropic viruses including variants of HIV); brain imaging (e.g., CT, MRI and SPECT); electrophysiology (e.g., computerized EEG analysis); development of new diagnostic methods; neuropsychological evaluation of psychosis and dementia; and drug trials in schizophrenia and other neuropsychiatric disorders.

The program invites applications from physicians and scientists for Medical Staff Fellow ($32,000), Research Staff Fellows ($20,000-$38,000), or Visiting Fellow ($20,000-$23,000) positions. Staff Fellows must be U.S. citizens with a doctoral degree. Initial appointments are for two years with further renewals possible. Visiting Fellows may be resident or non-resident aliens with a doctoral degree and no more than three years of relevant post-graduate experience. Visiting Fellows are appointed for one year with possible renewals for two additional years.

Interested candidates should send a curriculum vitae, a brief statement regarding experience and research interests, and the names of three references to:

Rex Cowdry, M.D.
Deputy Director for Research
NIMH Neurosciences Center at Saint Elizabeth's
2700 Martin Luther King, Jr. Avenue, SE
Washington, D.C. 20032
(202) 373-6200

(NW3251)E

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INTERNATIONAL CANCER RESEARCH FELLOWSHIP

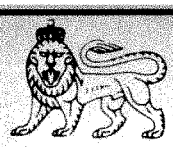
The Hayashibara Mutual Aid Fund, a non-profit making organization within the Hayashibara Group announces a fellowship program. Two awards will be made annually. Fellowship will be taken up at the newly opened Fujisaki Cell Center which is devoted for the basic and applied research related to the problems in human cancer. The fellowship is normally made for one year and is renewable for up to five years. The fellowship will be expected to commence within six months of the announcement of the awards. The awards are for high quality research work in one of the three categories described below.

1. Fundamental Leukemia-Lymphoma Research,
2. Cytokine-Lymphokine Research,
3. Hematopoietic Cell Cultures.

Qualified person who has a Ph.D., an M.D. or equivalent qualifications will be awarded on merit to suitably qualified research proposal and experiences. Additional information and application forms will be obtained from: **Jun Minowada, M.D., Director, Fujisaki Cell Center, 675–1,Fujisaki, Okayama 702, Japan.** (W5829)E

SMITHSONIAN POSTDOCTORAL FELLOWSHIPS IN MOLECULAR EVOLUTION AND PLANT PHYSIOLOGY

Postdoctoral Fellowships in Molecular Evolution and Plant Physiology are available for research to be conducted at two or more of the following Smithsonian Institution bureaus: National Museum of Natural History (Washington, D.C.); National Zoological Park (Washington, D.C.); Smithsonian Tropical Research Institute (Panama); and Smithsonian Environmental Research Center (Maryland). Stipends are $24,000 annually, including travel. Tenures are from 12 to 14 months. DEADLINE: May 1. Notification: August 1. For further information and application forms write: **Office of Fellowships and Grants, Desk M, Suite 7300, 955 L'Enfant Plaza, Smithsonian Institution, Washington, D. C. 20560.** (NW3262)E



KING'S COLLEGE LONDON
(University of London)

MRC Medical Research Council

Research Studentships
Biomolecular Sciences Division & MRC Cell Biophysics Unit

Applications are invited from final-year undergraduates (and others appropriately qualified) in departments of biochemistry, molecular biology, immunology, chemistry, physics and related disciplines for research studentships in the various sections of the Division and in the MRC Cell Biophysics Unit. In addition, four of the following research projects will be guaranteed a studentship, which will be allocated early:

Biophysics (Drury Lane) & Immunology (Kensington) Sections
Chromatin structure and the control of transcription
The structural basis of muscle contraction

Biochemistry Section (Strand & Kensington)
Membrane stability at low temperatures
Signal transduction mechanisms regulating lipolysis in intact and permeabilised erythrocytes
Neurotoxic amino acids, protein metabolism and cellular degeneration
Mechanisms of tissue damage in arthritis and in cardiac disease
The use of bacterial expression vectors to study gene expression in differentiating germ cells
Advanced spectroscopic studies of transition-metal centres in enzymes

Prospective candidates must obtain the details before applying and these may be freely obtained as follows:

For Biochemistry from:

Dr M Cannon, Biochemistry Department, King's College London, Strand, London WC2R 2LS (Telephone: 01-836 5454, extension 2674 FAX 01-836 5454, extension 2400)

For Biophysics, Immunology and the MRC Unit from:

Miss P A Wright, Biophysics Laboratories (King's College London, 26-29 Drury Lane, London WC2B 5RL (Telephone: 01-836 8851; FAX: 01-497 9078)

(8285)F

LUDWIG INSTITUTE FOR CANCER RESEARCH
MIDDLESEX HOSPITAL/UNIVERSITY COLLEGE BRANCH

PhD Studentship

The Institute is a superbly equipped Research Laboratory (Director Dr M D Waterfield) located in a newly refurbished building close to the Middlesex Hospital. There are seven groups working in the general areas of growth control and cell signalling in relation to carcinogenesis, differentiation and development. PhD Studentships are available beginning in October 1989. The group leaders and their research interests are as follows:—

PETER PARKER: the structure and function of signal transducing molecules and their role in 'normal' and neoplastic growth.

JEREMY BROCKES: molecular basis of cell patterning in limb development and regeneration.

MICHAEL WATERFIELD: structure and function of growth factors and receptors.

PAUL STROOBANT: purification and analysis of growth and differentiation factors, and their role in initiation, growth and metastasis of tumour cells.

MARK NOBLE cellular and molecular control of development, regeneration and neoplasia in the central nervous system; oncogene function and regulation of differentiation.

JIM WOODGETT: analysis of growth regulatory molecules: emphasis on regulators of oncogenes and transducers of growth factors.

PARMJIT JAT: molecular regulation of cell growth, immortalisation, transformation and senescence.

Interested students should apply as soon as possible to:
SECRETARY TO THE ADMINISTRATOR
LUDWIG INSTITUTE FOR CANCER RESEARCH
91 RIDING HOUSE STREET
LONDON W1P 8BT
Tel: 01-436 8441

(8223)F

Graduate Assistantships Molecular Biology and Biochemistry Wesleyan University Middletown, Connecticut 06457 USA

Several teaching and research assistantships are available in the Department of Molecular Biology and Biochemistry. Research interests of the faculty include nuclear magnetic resonance spectroscopy of proteins and nucleic acids; protein structure, function and design; protein-nucleic acid interactions and physical mechanisms of gene expression; statistical thermodynamic and computer modelling of biological processes; DNA replication; structure and function of chromosomal proteins; yeast genes homologous to protein kinase oncogenes; regulation of gene expression in embryogenesis and development and chemistry of reactions at phosphorus. All students receive a twelve month stipend, tuition remission, good fringe benefits and dependency allowance if applicable. For further information write: **Graduate Admissions, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06457 or call (203) 347-9411 X 2524.**

(NW3256)F

continued on page 30

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF CLINICAL BIOCHEMISTRY

TWO RESEARCH STUDENTSHIPS including **THE ELLIE BARKER RESEARCH STUDENTSHIP** funded from industrial and private sources are available for 3 years from 1st October, 1989. Each provide a personal grant of £4,000 per annum. One is to study the molecular cell biology of membrane traffic pathways in human cells with Dr J. P Luzio and the other to study the molecular biology of androgen — responsive genes of human hair follicles with Dr G. T. E. Kealey. Potential applicants with, or expecting to obtain, at least upper second class honours degrees should write for further particulars to **Dr. Luzio or Dr. Kealey at the Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR.** (8302)F

UNIVERSITY OF SUSSEX

RESEARCH STUDENTSHIPS

Applications are invited for two Ph.D. studentships available in October 1989 for candidates who have, or expect to obtain, first or upper second class degrees and are highly motivated towards research.

1) EUKARYOTIC MOLECULAR BIOLOGY in the laboratory of Dr. Alan Lehmann in the MRC Cell Mutation Unit. The successful applicant will join an active research group working on the cloning of human and other eukaryotic DNA repair genes. Applications including a c.v. and the names of two referees should be sent to: **Dr. A.R. Lehmann, MRC Cell Mutation Unit, University of Sussex, Brighton BN1 9RR (tel. 0273-678122)** from whom further details can be obtained.

2) MOLECULAR MICROBIOLOGY in the laboratory of Dr. Brian G. Spratt in the Microbial Genetics Group. An SERC Antibiotics/Recombinant DNA Initiative Ph.D. Studentship is available to work on the molecular basis, or molecular epidemiology, of penicillin resistance in clinical isolates of *Streptococcus pneumoniae.* A c.v. and the names of two academic referees should be sent to **Dr. Brian G. Spratt, Microbial Genetics Group, School of Biological Sciences, University of Sussex, Brighton BN1 9QG (tel. 0273-678309)** from whom further particulars can be obtained.

Applications for these studentships should be received by 10th March 1989. (8313)F

Cancer Research Campaign

POSTGRADUATE RESEARCH STUDENTSHIPS

As part of its continuing support for Cancer Research, The Cancer Research Campaign (CRC) has available the following PhD studentships to work within CRC laboratories and research groups. Studentships will be available from 1 October 1989. *In addition to the normal Research Council stipend the CRC studentships carry an additional £1,000 and full running expenses in support of the research.* Applications are invited from recent graduates or from final year undergraduates in relevant subjects — normally applicants should hold or expect to gain a first or upper second class honours degree.

CRC LABORATORIES, UNIVERSITY OF NOTTINGHAM — 2 studentships
Colorectal cancer antigens — **Professor R W Baldwin**
Idiotypic antibodies in immunoregulation — **Professor R W Baldwin**

CRC EXPERIMENTAL CANCER CHEMOTHERAPY RESEARCH GROUP, ASTON UNIVERSITY, BIRMINGHAM — 2 studentships from:
DNA sequence-specific recombinant molecules — **Professor M F G Stevens**
Membrane signalling-protein kinase C isoforms — **Dr A Gescher**
Tumour cell differentiation (extracellular matrix effects) — **Dr J A Hickman**
Tumour-host relationships (cytokines responsible for weight loss) — **Dr M J Tisdale**

CRC DEVELOPMENTAL TUMOURS RESEARCH GROUP, UNIVERSITY OF OXFORD — 2 studentships
Genetic analysis of teratocarcinoma cell differentiation — **Dr M Pera**
Regulation of IGF-11 gene expression — **Professor Graham FRS**

CRC FORMULATION UNIT, UNIVERSITY OF STRATHCLYDE — 1 studentship from:
Formulation of stable i.v. suspensions and emulsions of insoluble compounds or prodrugs — **Dr W R Vezin**
Prediction of solubility of slightly soluble compounds using molecular graphics — **Dr W R Vezin**

CRC LABORATORIES, DEPARTMENT OF CANCER STUDIES, UNIVERSITY OF BIRMINGHAM — 1 studentship from:
Antigenic epitopes of Epstein-Barr virus latent proteins analysed by T-cell cloning — **Professor A B Rickinson**
Characterisations of adenovirus E1 proteins responsible for malignant transformation — **Professor P H Gallimore**
T-cell receptor gene rearrangements and leukaemogenesis in ataxia telangiectasia patients — **Dr A M R Taylor**

CRC HUMAN CANCER GENETICS RESEARCH GROUP, UNIVERSITY OF CAMBRIDGE — 1 studentship
Molecular genetics of inherited cancer — **Dr B A Ponder**

BEATSON INSTITUTE FOR CANCER RESEARCH, GLASGOW — 3 studentships
The use of transgenic mice in studies on oncogenesis and differentiation — **Dr A Balmain**
Cell interaction via gap junctions in normal and neoplastic tissues — **Drs Finbow and Pitts**
Purification of novel growth factors from transformed cells — **Dr B Ozanne**

INSTITUTE OF CANCER RESEARCH, SUTTON, AND LONDON — 3 studentships
Mechanisms of *ras* oncogene transformation — **Dr C Marshall**
Sensitivity of human tumour cells to radiation: underlying molecular mechanisms — **Drs G Steel and T Macmillan**
Synthesis of some S-adenosyl-L-homocysteine derivatives and their nucleoside precursors — **Drs M Jarman and P Serafinowski**

PATERSON INSTITUTE FOR CANCER RESEARCH, MANCHESTER — 5 studentships
Growth factors in intestinal cells — **Drs G S Evans and M Mackett**
Cloning of mammalian and viral DNA repair genes — **Drs E Littler and G P Margison**
Novel anticancer drugs that disrupt microtubules — **Dr A McGown and Professor B W Fox**
Cell surface markers in cervical neoplasia — **Dr P L Stern**
Cellular adhesion and extracellular matrix in haemopoiesis — **Professor T M Dexter and Dr J T Gallagher**

CRC GRAY LABORATORY, NORTHWOOD, MIDDLESEX — 1 studentship
Studies of DNA Repair Processes — **Professor J Denekamp**

Candidates should write indicating in which project they are interested. Letters including a C.V. and names and addresses of 2 academic referees should be sent to:—

MRS J EASTWOOD (STUDENTSHIP SECTION)
CANCER RESEARCH CAMPAIGN,
2 CARLTON HOUSE TERRACE
LONDON, SW1Y 5AR

(8300)F

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF BOTANY
SERC funded PhD Studentship

Applications are invited for a 3-year PhD studentship to work on the purification of a chlorophyll biosynthesis enzyme from peas. The ultimate aim is to isolate cDNA and genomic clones for the enzyme, in order to study its expression. Applicants should have a good Honours degree in Biochemistry, Molecular Biology or a related subject. The studentship, which is funded by the SERC, will start as soon as possible, and in any case before 11th May 1989.

Applicants should send curriculum vitae and the names of two referees to Dr A G Smith, Department of Botany, University of Cambridge, Downing Street, Cambridge CB2 3EA, by **24th February 1989.**

(8257)F

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF BOTANY

PhD Studentships

The department offers research training leading to the degree of PhD in a number of areas of plant biology. Studentships in the following disciplines are funded by the SERC:

Regulation of Plant Metabolism **Chloroplast Molecular Biology**
Genetics and Molecular Biology of Plant-Pathogen Interactions
Plant Ultrasctructure **Ion Transport in Plants** **Guard Cell Physiology**
Plant Taxonomy **Experimental Mycology** **Developmental Biology**
Molecular Genetics of Chlorophyll Synthesis

Supervisors include: *Dr T ap Rees, Dr D Briggs, Dr P Echlin, Dr JC Gray, Dr DE Hanke, Dr HJ Hudson, Dr DS Ingram, Dr K Johnstone, Prof EAC MacRobbie, Mr PD Sell, Dr AG Smith and Dr PF Yeo.*

Applications are invited for studentships in the above areas of research, tenable from October 1989. Further details of the projects available, and application forms, may be obtained from Dr AG Smith, Department of Botany, University of Cambridge, Downing Street, Cambridge CB2 3EA, to whom applications should be sent by **6th March 1989.** Information regarding research projects in Tropical and Temperate Ecology, Physiological Ecology and Quaternary Research can be obtained from the same source. (8258)F

University of Leicester
Department of Biochemistry

CELLTECH RESEARCH STUDENTSHIP

Applications are invited for a special postgraduate research studentship which has been created by Celltech, Europe's foremost independent biotechnology company. The research programme will involve close collaboration between University scientists and their counterparts at Celltech, and will be focused on a study of factors influencing protein stability and the susceptibility to degradation by proteolytic enzymes *in vivo* and *in vitro*. Candidates should have, or expect to receive, a first or upper second-class honours degree (or equivalent qualifications) in a relevant subject and practical experience in enzymology. The appointment will be for a period of 36 months at a (tax-exempt) stipend of £3,500 *per annum*. In addition the award includes a travel allowance and the payment of University fees for registration for study for the PhD degree (UK and EEC nationals only).

Applications with the names of two referees should be send to Professor W. V. Shaw,
Department of Biochemistry, University of Leicester,
Leicester LE1 7RH. (Tel: 0533 523469).

CELLTECH

(W5838)P

UNIVERSITY OF MANCHESTER
DEPARTMENT OF CHEMISTRY
GLAXO RESEARCH STUDENTSHIP IN SYNTHETIC ORGANIC CHEMISTRY

Applications are invited for a research studentship to work with Professor E.J. Thomas on the total synthesis of a group of complex biologically active macrocyclic compounds. Applications should have, or expect to have, at least a 2.1 honours degree in chemistry. The post is funded by Glaxo Group Research and the stipend will be at the postgraduate studentship level, i.e. £3125 at present. Applications including a c.v. and the names and addresses to two referees should be sent as soon as possible to

Professor E.J. Thomas, The Department of Chemistry, The University, Manchester M13 9PL, from whom further particulars may be obtained. (8218)F

APPTS. WTD

Physicist seeking researcl teaching position. Willing cation. Experience in Ne Particle Ultrasonic Gr tional physics Vahé Melk Vanantyanz MSc Inns Höttinger Gasse 32, 6020 bruck, Tirol, Austria.

(W581

MARINE BIOLOGIST/Fish Developer (MSC, MBA) wi years Research and Ma ment experience seeks a tion in Fisheries or Aquacu any location (Research, elopment, management Marketing). Box No: 830 c/o 'Nature' 4 Little Esse London WC2R 3LF. (830

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nature

Please mention when replying to these advertisemen

ASSISTANTSHIPS

UNIVERSITY OF DURHAM
DEPARTMENT OF CHEMISTRY
SENIOR POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a Senior Postdoctoral Research Assistantship, tenable forthwith for a period of at least one year, to work with Dr. M. R. Bryce and Dr. D. Parker on a SERC supported project in collaboration with Cookson Group PLC. Candidates should have experience either in synthetic chemistry or in polymer chemistry.

Informal enquiries may be made by telephoning (091) 374 3133 or 3118.

The salary will be on the RA IA scale within the range £9,865–£11,680.

Further particulars may be obtained from the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE (Tel. No. (091) 374 2263)** to whom applications (three copies), including the names of three referees, should be sent not later than *Friday, 10th March, 1989* (quoting ref. C2 stating title of post).

(8295)P

UNIVERSITY OF DURHAM
DEPARTMENT OF PHYSIC
POSTDOCTORAL SENIOR RESEARC ASSISTANTSHIP

Applications are invited fo SERC funded Postdoctoral Ser Research Assistantship tena for three years from 1st Ap 1989 for work in Observatio Cosmology. Main interests the Cosmology Research Gro are in the determination of large scale structure of universe and in the formatic clustering and evolution of ga xies and of QSO's.

Informal enquiries may be ma to Dr. R. Fong (Tel. No. (091) 3 2157).

The salary will be on the RA scale within in the range £9,86 £11,680.

Further particulars may obtained from the **Registr Science Laboratories, Sou Road, Durham, DH1 3LE (Tel. N (091) 374 2263)** to whom applic tions (three copies), includir the names of three referee should be sent not later th *Friday, 17th February, 19* (quoting ref. P3 stating title post). (8303)P

NORDISK UK GRANT 1989

APPLICATIONS ARE INVITED FOR THE SEVENTH NORDISK UK GRANT

The Grant is intended to further research within the field of insulin-dependent diabetes and growth disorders in the United Kingdom. Awards for clinical and non clinical research are intended mainly as starter funding, usually for younger researchers, rather than covering the full-time salaries of research workers.

The Grant is given by Nordisk Gentofte A/S, the manufacturing division of Nordisk Insulinlaboratorium, a non-profit Foundation by Danish Royal Charter established in 1923.

The value of the 1989 Grant is £25,000 in total.

The successful applicants will be selected by an independent Board. It is unlikely that any single award will exceed £10,000.

For an application form and copy of the Statutes of the Grant please contact:

Nordisk UK Grant, c/o Nordisk-UK,
Nordisk House, Garland Court, Garland Road,
East Grinstead, West Sussex RH19 1DN

The closing date for receipt of completed applications is 22 March 1989.

(8306)H

THIS GRANT IS ADMINISTERED AS PART OF THE NORDISK-UK PROFESSIONAL PROGRAMME

WORKSHOPS

EMBO WORKSHOP

ILLEGITIMATE RECOMBINATION

MAY 28-31 1989 — PARIS, FRANCE

The goal of the workshop is to promote communication between scientists interested in mecanisms and consequences of recombination between DNA sequences of short or no homology.

Among the invited speakers are:
B. ALBERTS (USA), W. ARBER (SWITZERLAND), D.E. BERG (USA), S. BRON (THE NETHERLANDS), J. CHAMPOUX (USA), M. CHANDLER (FRANCE), C. M. CROCE (USA), R. DEONIER (USA), K. DRLICA (USA), H. IKEDA (JAPON), R. KAHMAN (FRG), R. KOLODNER (USA), A. LANDY (USA), D. LEACH (GB), D. LILLEY (GB), B. LLYOD (UK), D. MATHIEU-MAHUL (FRANCE), S. W. WATSON (USA), C. McHENRY (USA), M. RADMAN (FRANCE), L. RIPLEY (USA), R. T. SCHIMKE (USA), R. SCHMITT (FRG), H. SCHREMPF (FRG), F. STAHL (USA), J. WILSON (USA).

The number of participants will be strictly limited to 60. The registration fee will be 500 French Francs. A limited number of fellowships may be available for junior scientists. Applications including CV and research interests should be sent before April 1st, 1989 to **Dr S. D. EHRLICH, LABORATOIRE DE GENETIQUE MICROBIENNE, INRA-DOMAINE DE VILVERT, 78350 JOUY EN JOSAS, FRANCE (Tel: 33-1 34 65 25 10, Fax: 33-1 34 65 22 73).**

(W5834)V

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Request for Applications

GENE THERAPY AND CYSTIC FIBROSIS

The Cystic Fibrosis Foundation invites investigator-initiated grant applications on the fundamental scientific issues associated with applying gene therapy to cystic fibrosis (CF).

This request is intended to encourage innovative and meritorious projects that can advance the study of CF gene therapy while the search for the CF gene continues. Research areas responsive to this request include, but are not limited to:

- Identification of appropriate airway cells for therapeutic replacement of defective CF genes;
- Development and risk assessment of vector systems for delivering the normal allele of the CF gene into airway cells;
- Development of model systems for examining the scientific, safety and technical issues associated with CF gene therapy.

Research approaches and models must be directly relevant to the application of gene therapy to CF. Applicants may request support for up to three years; funding levels will be commensurate with the scope of projects.

The deadline for submission of applications is **July 1, 1989.** For further information and application forms, please contact **Dr. Robert J. Beall** or **Ms. Dale Glass** at the

Cystic Fibrosis Foundation

6931 Arlington Road
Bethesda, MD 20814
(301) 951-4422

(NW3257)H

UNIVERSITY OF WALES COLLEGE OF MEDICINE TENOVUS INSTITUTE FOR CANCER RESEARCH AND UNIVERSITY OF LEICESTER

(DEPARTMENTS OF ANATOMY AND MOLECULAR BIOLOGY) RESEARCH SCHOLARSHIP

Required for studies on the regulation of growth factor gene expression by steroid hormones during development. The position is for 3 years leading to a higher degree. Candidates should have a good degree in a relevant biological science and have an interest in molecular biological and endocrinological aspects of development and proliferation. The successful applicant will have an unique opportunity to enjoy the facilities of the Molecular Endocrinology laboratory of the Tenovus Institute and the above named departments of the University of Leicester and gain extensive experience in technologies related to steroid-hormone mechanisms, protein-DNA interactions and cloning and analysis of genes.

Applications in the form a curriculum vitae with the names and addresses of two referees to **Dr Peter Davies, Tenovus Institute for Cancer Research, Heath Park, Cardiff** from whom further information is available. (8274)H

AWARDS

NUFFIELD FOUNDATION

Awards to Newly Appointed Science Lecturers in Science, Applied Science and Engineering

The Trustees of the Nuffield Foundation are offering a limited number of awards of up to £4,000 to newly appointed University and Polytechnic lecturers in Science, Applied Science and Engineering.

The object of the awards is to give assistance to such lecturers in the early stages of their independent research careers.

Awards will be made competitively and preference will be given to lecturers wishing to explore novel and original lines of research that may not yet be at a stage suitable for Research Council support. Sixty awards were made in 1988.

ELIGIBILITY

Applicants must be newly appointed to lectureships or equivalent posts in Science, Applied Science, Mathematics, Engineering or Medical Departments. Candidates must apply within 12 months of taking up their posts and may apply between the time of being appointed and taking up the post.

HOW TO APPLY

There are two selection meetings a year. The next closing date for applications is 31st March 1989. Applications must be on the appropriate form, which is available from the **Nuffield Foundation, 28 Bedford Square, London WC1B 3EG.**

The scheme is only open to applicants from institutions within the United Kingdom. (8262)N

MOUNT ST. MARY'S COLLEGE
SPINKHILL
SHEFFIELD

POST GRADUATE AWARD

An award of not less than £2,000 per annum for two years is available for former pupils of the College who are in need of support for a Post Graduate course at a U.K. University.
Forms of application are available from the Clerk to the Trustees, 5 Porlock Grove, Trentham, Stoke-on-Trent ST4 8TN. (8320)N

ASSISTANTSHIPS continued

BIRKBECK COLLEGE
(University of London)

POSTDOCTORAL RESEARCH ASSISTAN

DEPARTMENT OF CRYSTALLOGRAPHY

Applications are invited for a postdoctoral research assistantship fun by the SERC for 3 years. The project involves the modelling of prot solvent and protein-in-protein interactions using state of the art elec static models. An essential part of this project will involve the Resea Assistant collaborating with Dr. S. Price in the Theoretical Chemis Department, Cambridge as well as with Dr. J. M. Goodfellow and Dr. J. Thornton at Birkbeck. Experience in computational techniques with a ba ground in a physical or chemical science would be an advantage. Sal will be within range £11515–£13330 p.a. inclusive of London Weighting Applications in duplicate including a full curriculum vitae and names a addresses of 2 referees should be sent to the **Assistant Secretary (Pers nel), Birkbeck College, Malet Street, London, WC1E 7HX.**

Further details can be obtained from Dr. Goodfellow on 01-631 6368. (8315)I

SEMINARS & SYMPOSIA

LEUKAEMIA RESEARCH FUND

International Research Symposium on "PRELEUKAEMIA"
at the Royal College of Physicians, London NW1
on Monday 13th March 1989

Registration fee (including lunch) £15
For programme details and registration form apply to:
LEUKAEMIA RESEARCH FUND, 43 Great Ormond Street, London WC1N 3JJ (tel: 01-405 0101) (8316)M

Third Forefront Symposium of the International Society of Nephrolog

"Organization of membrane polarity in epithelial cells
July 2 to July 6, 1989, Arolla, Switzerland

The general topic is "Organization of Membrar Polarity in Epithelial Cells". Subjects to be considere include differentation and extracellular matrix, polar zation of epithelial cells, biogenesis of membrar proteins, structure and function of transport protein housekeeping functions of epithelial cells and regul tion of epithelial cell functions.

Invited speakers and chairmen:
Q. Al Awqati; A. Aronson; D. Ausiello; D. Benos; M Burg; M. Caplan; B. Cunningham; A. Doucet; I Ekblom; D. Fambrough; H. Garty; G. Giebisch; M Gottesman; S. Gluck; H. W. Harris; J. D. Horisberge T. Kleyman; R. Kopito; B. Lindemann; D. Louvard; Mauchamp; M. Mousseker; S. Nakanishi; W. Nelson; L. Palmer; O. Pongs; J. Pouyssegur; L. Reid; E Rodriguez Boulan; H. Sackin; M. Spiess; M. Welsch; E M. Wright.

Interested people should send their application (CV list of publications, 1 page abstract) to:

B. Rossier, M.D.
Inst. de Pharmacologie
Bugnon 27
CH-1005 Lausanne

Deadline: April 15, 1989. Selected applicants will b notified by the beginning of May.

Travel funds in limited amounts will be available fc students and postdoctoral fellows. (W5835)M

Royal Postgraduate Medical School

(University of London)

Hammersmith Endothelium Course

18-20th September 1989

Topics covered will include endothelial pharmacology, physiology, pathology and cell culture techniques

peakers include:

Professor E Anggård
Professor K Brigham
Professor G Burnstock
Dr D Crossman
Dr J Giddings
Dr C Haslett
Dr C Kluft
Dr J Martin
Professor A Maseri
Dr S Moncada
Dr J Pearson
Dr C Pusey
Dr A Rees
Dr T J Rink
Professor J Ritter
Professor U S Ryan
Professor T Williams

ourse organiser Dr J Warren

ourse fee (incl. catering) **£200**

ooking forms and rther details may obtained from: Wolfson Conference Centre
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 0NN

lephone: 01-740 3117

losing date r applications: 30th June 1989

(8261)C

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ve your information prob- the quick way with com- -aided literature searches ver 300 leading databases. l range of scientific subjects red". Box No: 8308D c/o ire' 4 Little Essex St. on WC2R 3LF. (8308)D

MISCELLANEOUS

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MSc/Diploma in Neuroscience
Department of Preclinical Veterinary Sciences
University of Edinburgh

The above internationally recognised Department together with Departments in the Faculty of Medicine offers a one year MSc in Neuroscience. The course is practically based and is by examination and dissertation. There is instruction on modern anatomical and physiological techniques used in Neuroscience. The techniques include intracellular and extra-cellular recording, voltage-clamp, patch-clamp and receptor binding. A five week clinical module is now included and covers Dementia, Brain Tumours and Depression.

Suitable applicants would include Honours Science students wishing to convert to Neuroscience or graduates of Medicine, Dentistry and Veterinary Science. A limited number of studentships are available.

Further details may be obtained by contacting **Dr R J Martin, Dept. of Preclinical Veterinary Science, R(D)VS, Summerhall, University of Edinburgh, Edinburgh EH9 1QH. Tel. 031 667 1011 Ext. 5344.** (8260)C

WACIID '89

Winter Advanced Course for Immunology and Infectious Diseases '89

NAGARA FURUSATO VILLAGE, CHIBA PREFECTURE, JAPAN
5th - 8th February, 1989

Organizing Chairman: Tomio Tada

Sponsored by:
Japan International Friendship and Welfare Foundation

This Course, the first in a series to be held in Japan, will bring together internationally-recognized experts in the fields of Immunology and Infectious Diseases, as well as students actively engaged in research.

The program includes lectures, informal discussions and posters, as well as social and sports activities.

Invited speakers include:

Drs. F Alt (U.S.A.)
M Davis (U.S.A.)
S Habu (Japan)
Len Herzenberg (U.S.A.)
Lee Herzenberg (U.S.A.)
N A Mitchison (England)
K Rajewsky (F.R.G.)
A. Singer (U.S.A.)
T Tada (Japan)
M Taniguchi (Japan)
T Tokunaga (Japan)
and
M Cooper (U.S.A.)
H Grey (U.S.A.)
D. Littman (U.S.A.)
T Kishimoto (Japan)
G Mitchell (Australia)
K Okumura (Japan)
E Reinherz (U.S.A.)
M Steinmetz (Switzerland)
K Takateu (Japan)
T Taniguchi (Japan)
A Weiss (U.S.A.)
I Weissman (U.S.A.)

The Course terminates with an open symposium and a graduation party at the Sheraton Grande Tokyo Bay Hotel & Towers, Urayasu, Chiba Prefecture. Those who are interested in future meetings should make their enquiries to **Prof. Tomio Tada, Department of Immunology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113, Japan.** (W5806)C

1890-1990 CSH CENTENNIAL

Conference on

TRANSLATIONAL CONTROL

September 13-17, 1989

Organized by:

Dr. Michael B. Mathews, *Cold Spring Harbor Laboratory*
Dr. Nahum Sonenberg, *McGill University*

Topics for discussion will include:

- *growth and development* • *mRNP*
- *metabolic effects* • *heat shock* • *interferon*
- *animal, plant, procaryotic and viral systems*
- *mRNA structure* • *mRNA stability*
- *ribosome frameshifting* • *SRP*
- *convalent modifications of translation factors*
- *translation factor genes and their expression*

Supported by a grant from ICN Biomedicals, Inc.

Registration and abstract materials are available from:

Meetings Coordinator
COLD SPRING HARBOR LABORATORY
P.O. Box 100
Cold Spring Harbor N.Y. 11724
(516) 367-8345

Abstract deadline: June 23, 1989

(NW3248)C

FIFTH ANNUAL MEETING ON ONCOGENES

June 27–July 1, 1989

Hood College
Frederick, Maryland

Meeting organizers:
Peter K. Vogt & Raymond L. Erikson

For information contact:
Margaret Fanning
Conference Coordinator, FACS
P.O. Box 249
Libertytown, Maryland 21762
(301) 898-9266 FAX (301) 898-9173

(NW3255)M

INTERNATIONAL SYMPOSIUM ON SIGNAL, PERCEPTION AND TRANSDUCTION IN HIGHER PLANTS

NATO ADVANCED RESEARCH WORKSHOP TOULOUSE, FRANCE, 9-13th JULY 1989

The workshop will examine the most recent available data on molecular mechanisms involved in the response of plant cells to different biotic and abiotic stimuli.

Topics to be covered include:
Stimulus-response coupling mechanisms — Plant membrane receptors — Second messengers in plant — Message transducers in plants — Specific signalling devices in plants — New approaches based on DNA recombinant technology.

The workshop will consist of lectures, discussion and poster sessions. There is a limited member of NATO bursaries to assist post-graduate students from NATO countries and especially from Greece, Portugal and Turkey.

Details and applications (with a C.V.) to:

Dr. Raoul RANJEVA (Workshop Director)
Centre de Physiologie Végétale
URA CNRS 241
Université Paul Sabatier
118 route de Narbonne
31062 Toulouse Cedex FRANCE
Telephone: 61 55 67 53
Telefax: UNIPSAB 524880 F
Telecopy: 61 55 64 70

Deadline: 01/03/89

Organizing Committee: R. RANJEVA (Director, Toulouse); A.M. BOUDET (co-director, Toulouse); J. MORRE (USA); A. SIEVERS (FRG); A.J. TREWAVAS (UK). (W5828)M

ROYAL SOCIETY DISCUSSION MEETINGS
APRIL 1989

5 and 6 April: 'THE DEEP-SEA BED: ITS PHYSICS, CHEMISTRY AND BIOLOGY' organized by Professor H. Charnock, F.R.S., Professor J.M. Edmond, F.R.S., Professor I. McCave, Dr A.L. Rice and Dr T.R.S. Wilson.
Speakers: K.J. Richards, I.N. McCave, M. Angel, S. Emerson, T.R.S. Wilson, R.C. Aller, M. Rutgers van der Loeff, P.A. Jumars, A.R.M. Nowell, A. Gooday, R.J. Parkes, E.T. Sunquist, T.P. Gross and S.A. Thorpe.

26 and 27 April: 'GROWTH FACTORS IN DIFFERENTIATION AND DEVELOPMENT' organized by Dr M.J. Crumpton, F.R.S. and Professor T.M. Dexter.
Speakers: G.B. Pierce, J.M.W. Slack, T.M. Dexter, D. Metcalf, F.R.S., J.J.T. Owen, F.R.S., T. Honjo, M. Noble, A.B. Roberts, R. Ross, M. Bernfield, W.J. Leonard, R.H. Michell, F.R.S., M.J. Crumpton, F.R.S. and E. Rozengurt.

Registration details (no fee for attendance): The Scientific Meetings Secretary (ref. DMN/CAJ), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG (Tel: 01-839 5561, ext. 278. Telex: 917876). (8304)M

IMPERIAL CANCER RESEARCH FUND DNA TUMOUR VIRUS MEETING 1989

The Imperial Cancer Research Fund is sponsoring a meeting on DNA Tumour Viruses to be held in Churchill College, Cambridge, England from *24th to 29th July, 1989* (5 days). The subject of meeting will be the molecular biology of small DNA tumour viruses, particularly polyoma virus, SV40 and adenoviruses. It will replace for this year the annual Cold Spring Harbor DNA tumour virus meeting.

The meeting is open to all interested research workers. The scientific programme will include oral presentations and posters. The cost for registration, accommodation, food and the abstract book will be £260.

Anyone interested in attending the meeting should apply immediately for further details to: **Mrs. Clare Middlemiss, ICRF DNA Tumour Virus Meeting Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England.** (8124)M

EIGHTH SUMMER SYMPOSIUM IN MOLECULAR BIOLOGY

DNA–PROTEIN INTERACTIONS

The Pennsylvania State University JULY 26–JULY 28, 1989

KEYNOTE ADDRESS: Arthur Kornberg

STRUCTURE AND FUNCTION IN DNA-PROTEIN COMPLEXES
T. Lohman, R. Klevit, G. Ackers, A. McPherson

DNA-REPRESSOR MOLECULAR RECOGNITION
C. Pabo, R. Sauer, A. Aggarwal, K. Matthews

MECHANISMS OF DNA SYNTHESIS
C. Joyce, K. Johnson, C. McHenny, S. Goff

ENZYMATIC ASPECTS OF ENDONUCLEASES
P. Modrich, C. Frederick, P. von Hipple, L. Jen-Jacobson

This symposium is organized by the Penn State Cooperative Program in Biotechnology and is supported by the Industrial Affiliates of the Program.

For information on poster presentations and registration, contact:

Symposium Program Coordinator
Eighth Summer Symposium in Molecular Biology
208 South Frear Laboratory
The Pennsylvania State University
University Park, PA 16802
Telephone: (814) 863-3696

(NW3247)M

Labsystems LABSYSTEMS RESEARCH LABORATORIES

LABSYSTEMS RESEARCH SYMPOSIUM
RECOMBINANT SYSTEMS IN PROTEIN EXPRESSION
July 23.-26. 1989, Imatra, Finland

PROGRAMME

Key Note Speaker: Leroy Hood (Pasadena, CA)

SESSIONS

PROKARYOTIC SYSTEMS	G.G. Gause (Moscow); K.K. Stanley (Heidelberg); M. Uhlén (Stockholm)
LOWER EUKARYOTIC SYSTEMS	R.W. Davies (Ontario), S. & A. Kingsman (Oxford, UK); V.L. MacKay (Seattle); P. Valenzuela (Emeryville)
VIRAL VECTORS — DNA-VIRUSES	D.H.L. Bishop (Oxford, UK); L. Gissmann (Heidelberg); C. Baker (Bethesda, M,D); R. Kaufman (Cambridge, MA); P. Tiollais (Paris); M.D. Summers (Texas A & M)
RETROVIRAL VECTORS	W. Ostertag (Hamburg); I.M. Verma (San Diego)
EXPRESSION IN ANIMAL CELLS	S.A. Aaronson (Bethesda, MD); T. Honjo (Kyoto); A. Levinson (San Francisco); E. Ruoslahti (La Jolla); J. Schlessinger (King of Prussia, PA)
TRANSGENIC SYSTEMS	M.R. Capecchi (Salt Lake City); A.J. Clark (Edinburgh); B. Groner (Bern)

Organizing Committee:

Kari Alitalo	University of Helsinki
Marja-Liisa Huhtala	Labsystems Research Laboratories
Jonathan Knowles	Technical Research Center of Finland
Antti Vaheri	University of Helsinki

REGISTRATION AND INFORMATION:

Organizing Committee
Secretary: Ms. Aira Halonen
Labsystems Research Laboratories
Box 8, SF-0081 Helsinki, FINLAND
Tel. +358-0-782234, Fax +358-0-789 732

The symposium is o[limited] to 100 participants. Deadline for registration and possible poster or short communicati[on] submission is May 31, 1989. Registration fee 750 US$, after May 31 850 US$. Registration [includes] full accommodation, lectures and travel from Helsinki to Imatra [and] back. Registration forms should be send to the Organizing Committee.

(W5826)M

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SOME LIKE IT HOT:

Sequencing With TAQ DNA Polymerase

Stratagene's new ThermalBase™ DNA Sequencing Kit utilizes the thermostable DNA polymerase of thermus aquaticus (Taq polymerase). There are numerous advantages to sequencing with Taq polymerase.

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The reactions are carried out at 70°C, substantially reducing DNA secondary structure artifacts commonly seen with the Klenow fragment of *E.coli* DNA polymerase, reverse transcriptase, or modified T7 DNA polymerase (Sequenase*).

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The higher temperature also creates more stringent conditions for primer binding. Thus false priming is far less likely to occur at sites of imperfect hybridization.

The ThermalBase™ Sequencing Kit protocol is as simple as sequencing with the Klenow fragment or Sequenase*. Uniform labeling can be achieved with ^{32}P or ^{35}S nucleotides or oligonucleotide primers (including ^{32}P, ^{35}S, or fluorescent primers for automated systems). Single stranded or double stranded (boiling miniprep) DNA templates may be used.

*Sequenase is a trademark of United States Biochemical Corp.



Dr. Maryann Zounes of Syntro Corporation has independently compared Stratagene's Taq Polymerase with Sequenase* (modified T7 DNA polymerase) and a competitor's Taq polymerase. Double stranded supercoiled DNA was prepared using the boiling miniprep procedure. Sequenase* reactions were performed according to the manufacturer's instructions.

Arrows highlight Sequencing artifacts.

The accompanying figure illustrates the ability of Stratagene's Taq polymerase to sequence a double stranded DNA region in which Sequenase* and a competitor's Taq polymerase generate multiple artifacts (arrows). Stratagene's Taq polymerase proceeds through these regions with little difficulty. Overall, Taq polymerase provides substantially more information in artifact-prone regions than either the Klenow fragment or Sequenase*.

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11TH INTERNATIONAL CONGRESS OF THE INTERNATIONAL SOCIETY OF DEVELOPMENTAL BIOLOGISTS 20TH-25TH AUGUST 1989

JAARBEURS CONGRESS BUILDING
UTRECHT THE NETHERLANDS

YOUR OPPORTUNITY TO REVIEW ADVANCES IN THE FIELDS OF HUMAN, ANIMAL AND PLANT DEVELOPMENT

Developmental biology is a rapidly advancing science which enjoys increasing attention from a wide range of associated disciplines. The programme for this major scientific event will place emphasis on assessments in a wide variety of disciplines, including molecular biology, cell biology, biochemistry, embryology, genetics, immunology, botany, oncology and biotechnology. Plenary lectures, parallel symposia, poster contributions and special social events will combine to make the congress an international success.

Chairpersons		Symposia topics include:
R.G. Edwards	Cambridge	In vitro fertilisation and embryo transplantation
W.J. Gehring	Basel	Genetic control of pattern formation
Th. Graf	Heidelberg	Haemopoietic stem cell differentiation
J.B. Gurdon	Cambridge	Embryonic induction
J.K. Heath	Oxford	Growth factors and their receptors
M. Johnson	Cambridge	Cytoplasmic organisation and development
G. Klein	Stockholm	Developmental tumors
N. LeDouarin	Nogent-sur-Marne	Developmental neurobiology
A. McLaren	London	Germ cells
F. Melchers	Basel	Developmental immunology
H. Mohr	Freiburg	Developmental botany
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I.M. Verma	San Diego	Signal transduction and transcriptional regulation
E. Wagner	Heidelberg	Gene transfer into stem cells and embryos

International Exhibition: the publishers of *Nature* will be organising an associated international exhibition.

Satellite meetings: "Early amphibian development", contact person A.J. Durston
"Pathobiology of human germ cell tumors", organisers I. Damjanov, J.W. Oosterhuis, H. Walt
"Microgravity as a tool in developmental biology" organiser G.A. Ubbels.

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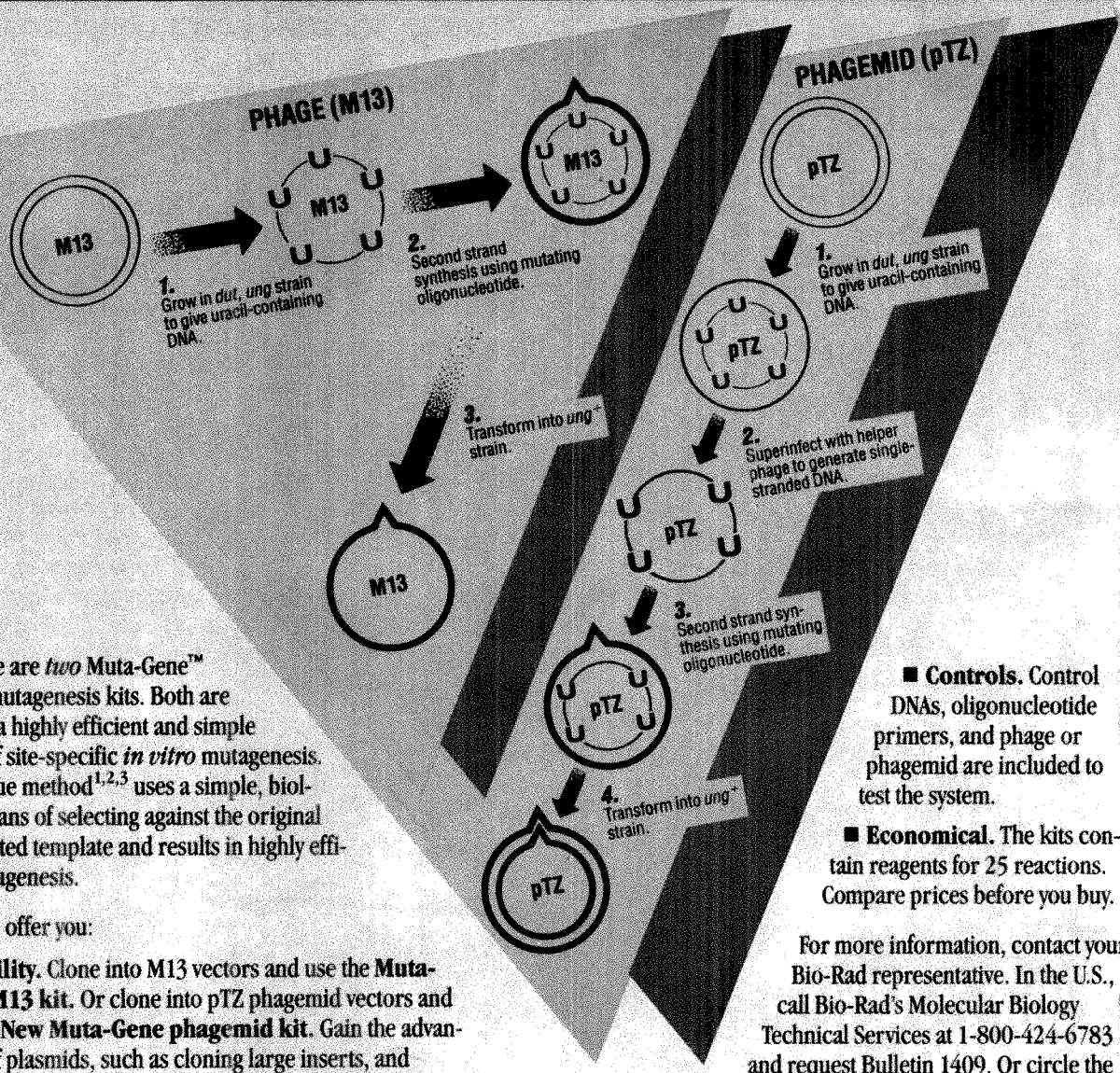
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*These phagemids were constructed from pUC18/19 by inserting the origin of replication.[4] Under most circumstances, they replicate as plasmids. Super infection with a helper phage results in phage-like particles containing single-stranded phagemid DNA.

[1] Kunkel, T.A. *Proc. Natl. Acad. Sci. USA* **82,** 488-492. 1985.
[2] Kunkel, T.A., Roberts, J.D., and Zakour, R.A. *Meth. Enzymol.* **154,** 367-382. 1987.
[3] Patent pending.
[4] Mead, D.A., Szczesna-Skorupa, E., and Kemper, B. *Prot. Eng.* **1,** 67-74. 1986.

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* Illustration by Tom Matthews

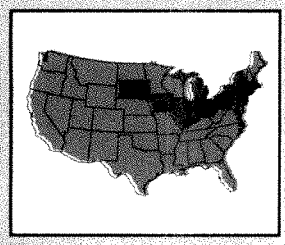
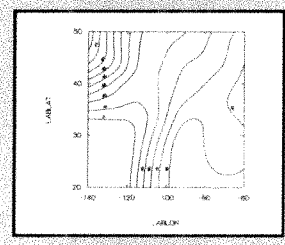
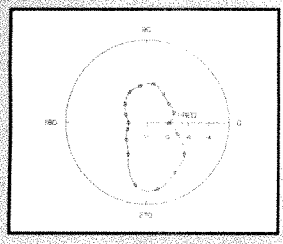
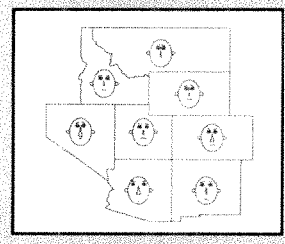
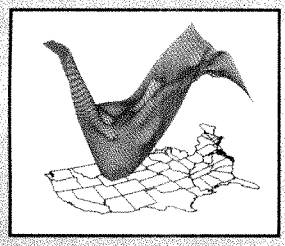
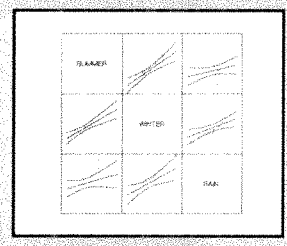
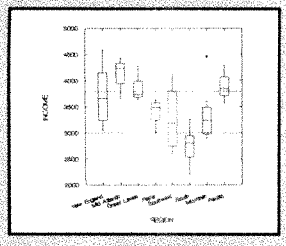
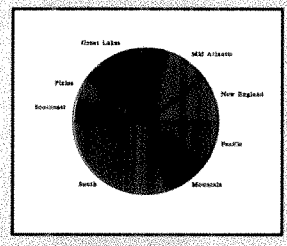
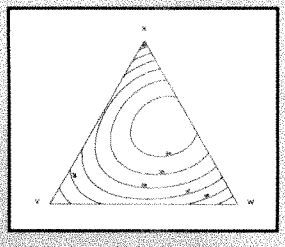
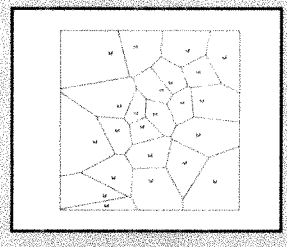
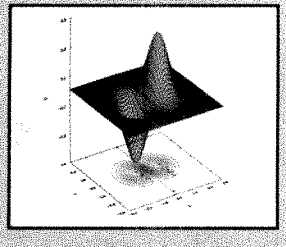
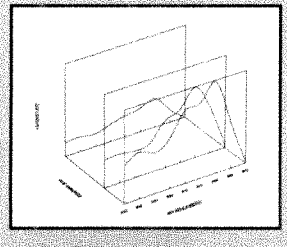
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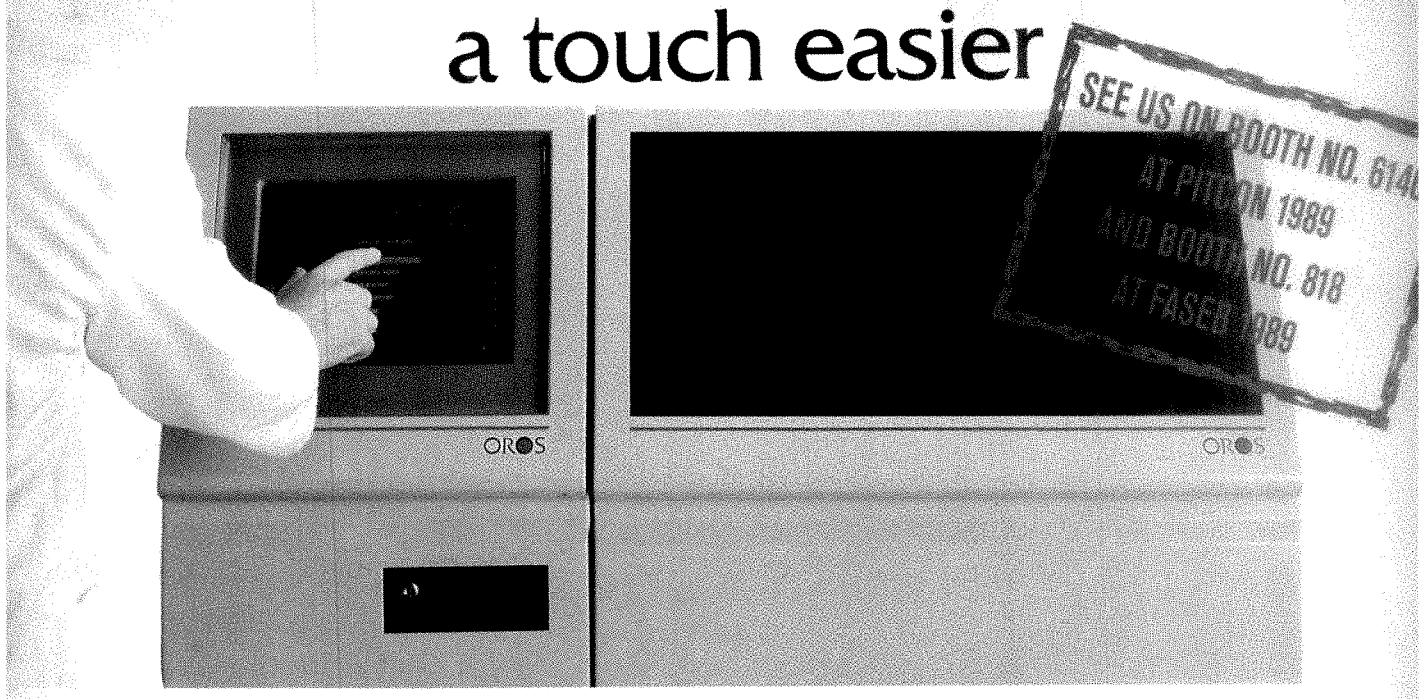
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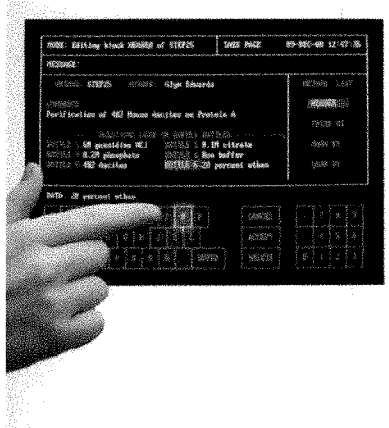
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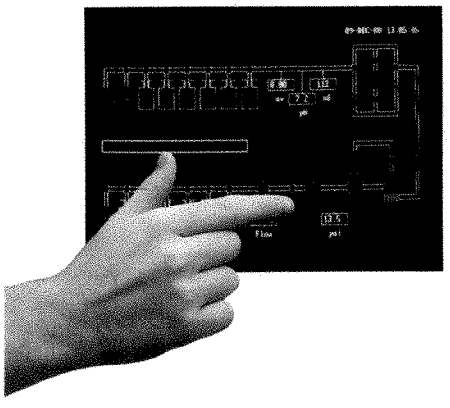
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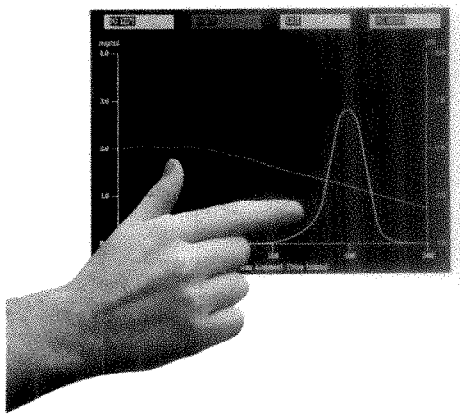
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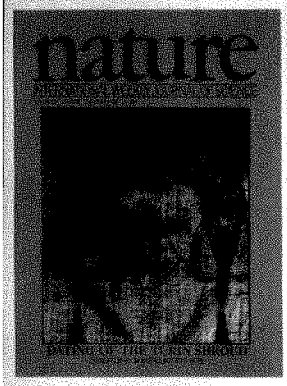
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nature

16 February 1989
Vol. 337 Issue No. 6208

A colour-enhanced image of the Shroud of Turin, produced at the Jet Propulsion Laboratory in Pasadena, California. For the carbon dating results, see page 611. For dissent and defence, page 594. Photo: Bernsen's International.

THIS WEEK

A pulsar is born

The pulsar now uncovered in the remains of supernova 1987A is not at all what was expected. It's spinning twice a millisecond, is apparently in a close 8-hour orbit with an unknown companion, and is underluminous for its rotation rate. All these pose theoretical difficulties. Page 595.

AIDS inhibitors

The crystal structure of HIV-1 protease confirms its relationship to cellular aspartyl proteases and should facilitate the design of inhibitors for use in AIDS therapy, pages 615 and 596.

Climate carbonates

Carbonate deposition is one factor that affects climate and atmospheric carbon dioxide concentration. A model for the dependence that distinguishes

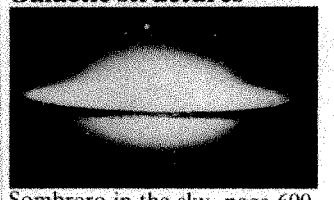


shallow-water and ocean-water carbonate deposition, described on page 637, suggests that it is the presence of plankton in the open ocean that has prevented continued glacial conditions on the Earth.

Extinction event

A period of diversification of the plankton of the Latest Proterozoic (about 600 million years ago) was followed by an extinction event at the Proterozoic/Cambrian boundary, pages 642 and 602.

Galactic structures



Sombrero in the sky, page 600. Here in the Solar System, the Earth has a 'ring', page 629.

Eruptions and implosions

The rare event of an earthquake occurring by implosion, rather than by sliding, is investigated further along the plate boundary in Iceland, recent site of such earthquakes, page 640. A massive volcanic eruption, larger than the devastating Lakigigur eruption of 1783, seems to have occurred on the Pacific sea floor in the 1960s. The 15 km^3 of erupted lava has only now been observed by sonar, leading to an eruption of new ideas, page 603.

Organelle assembly

A constitutive heat-shock protein, hsp60, is necessary for the correct assembly of multi-subunit mitochondrial enzymes in yeast (page 620), supporting the concept of protein-catalysed assembly of proteins entering the mitochondrial matrix.

Regulatory mechanism?

Two types of thyroid receptor protein are synthesized by alternative splicing from the rat *erbA*α gene; one of them does not bind thyroid hormone and inhibits the hormone-dependent action of the other *in vitro*, page 659.

Guide to Authors

Facing page 595.

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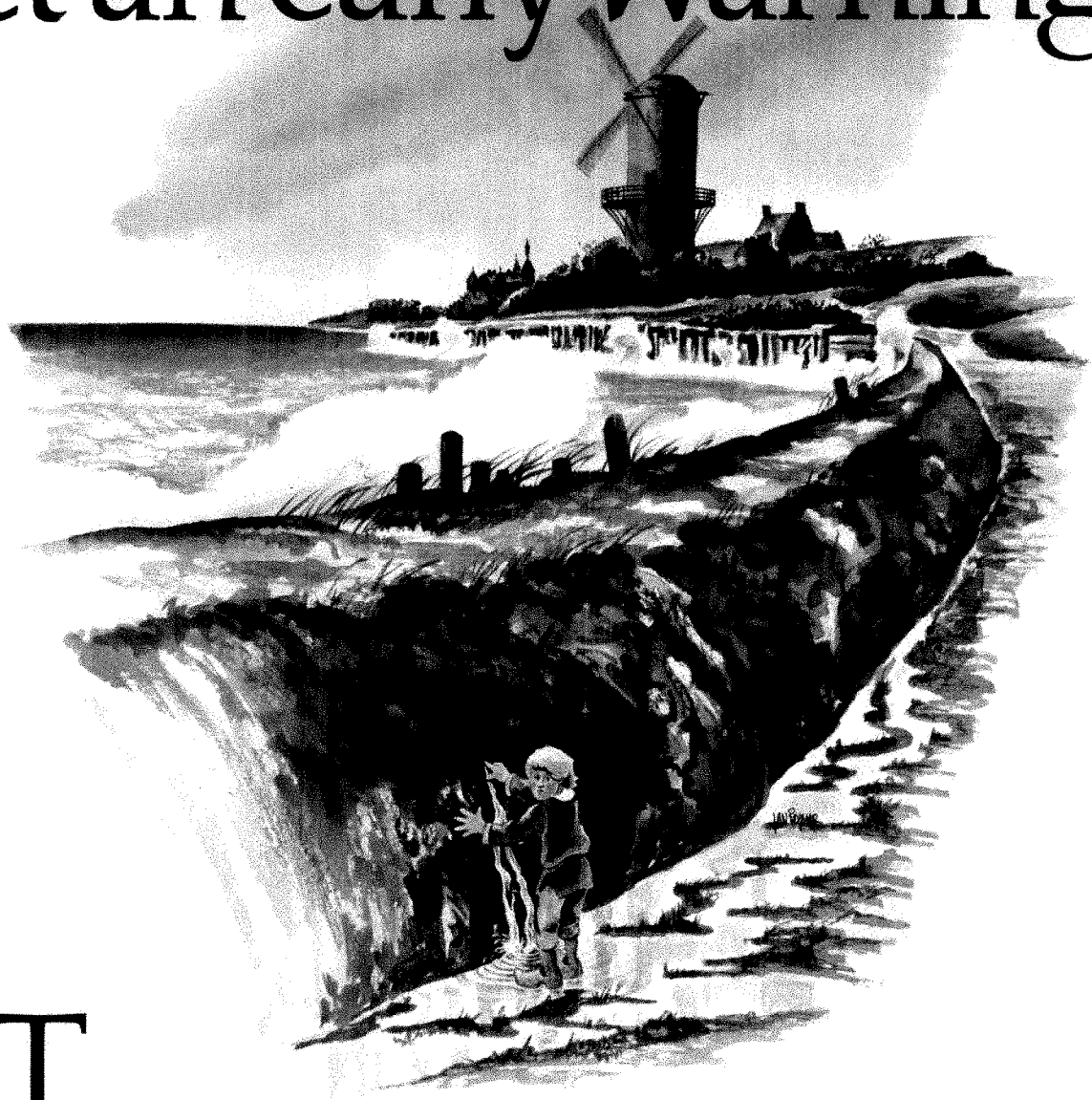
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The illustration depicts the little Dutch boy Hans Brinker saving his home-town of Haarlem from flooding by sticking his finger into a hole in the dyke. The story was written by Mary Mapes Dodge in 1873.

This legend about the fair-haired hero of Haarlem is one of the world's best known. As with all good fairy-tales, it has a moral: "Don't wait until too late."

Serum TPA measurements: For early and efficient detection of recurrent malignant disease.

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Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1
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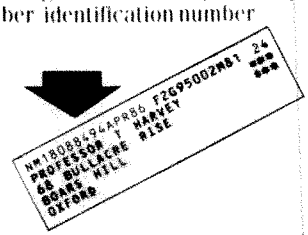
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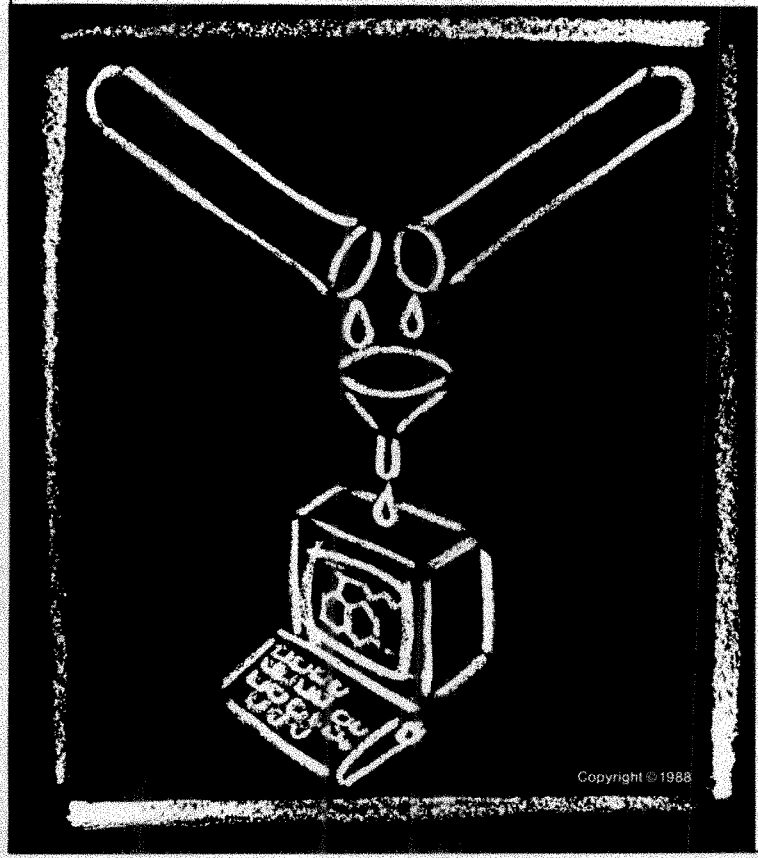
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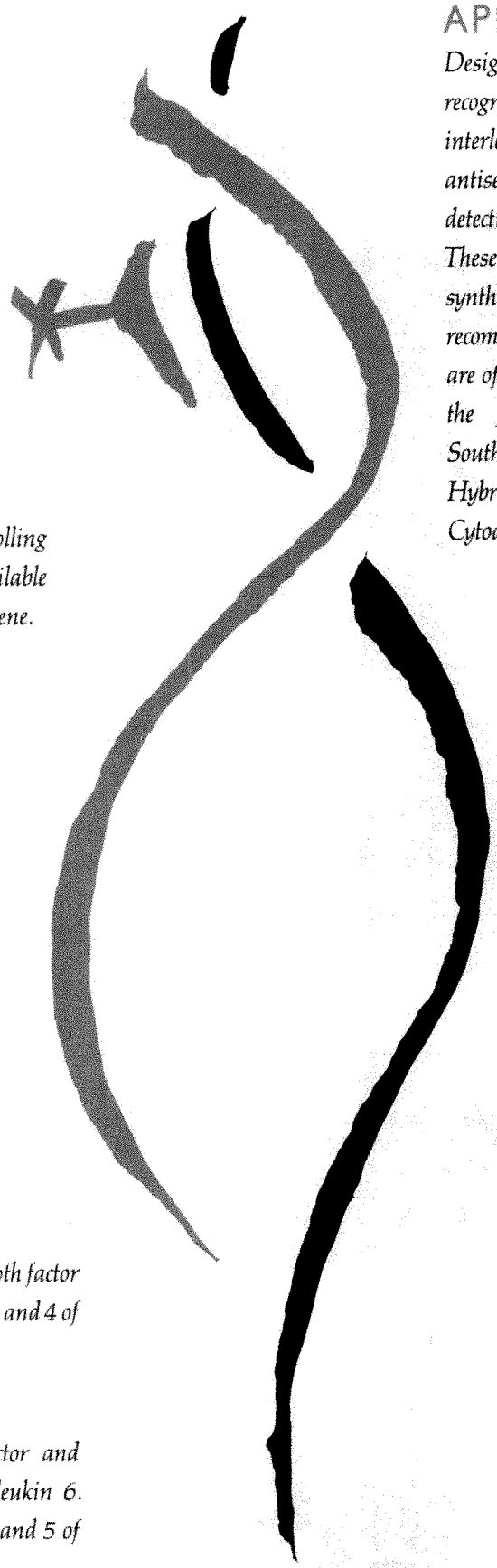
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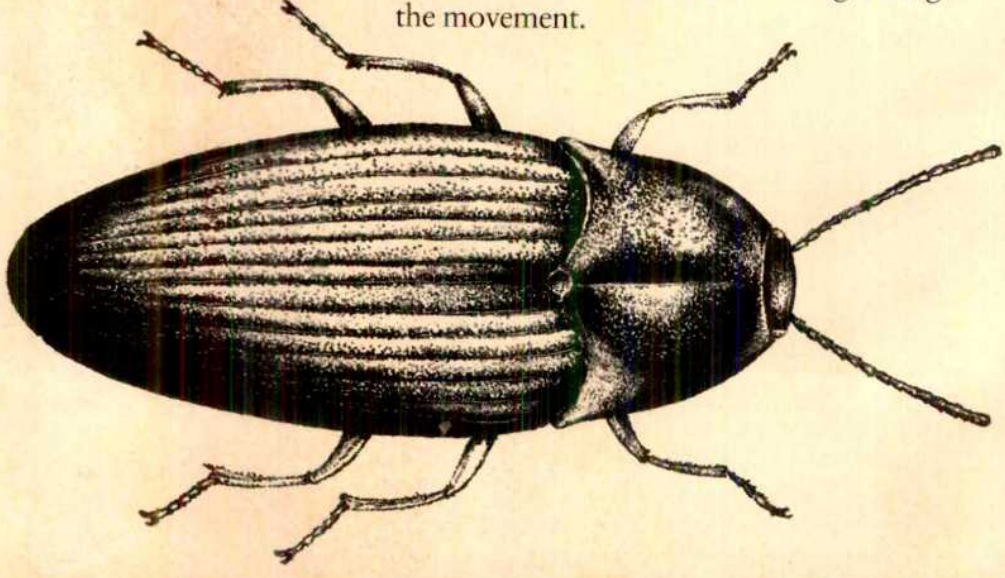
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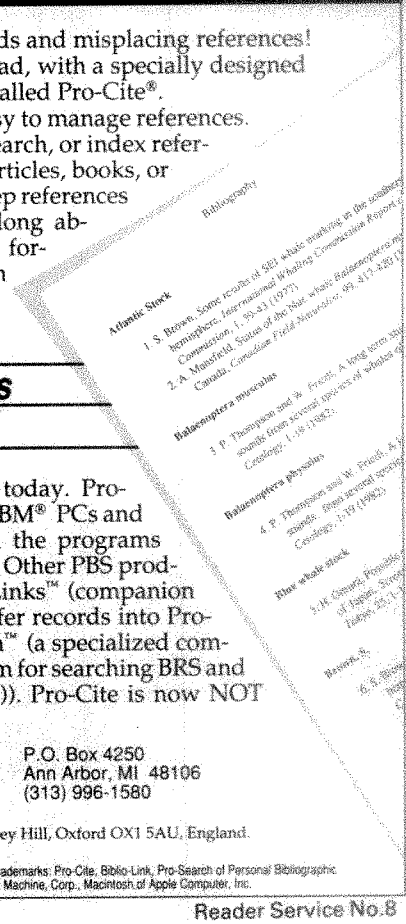
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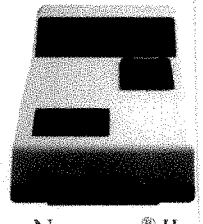
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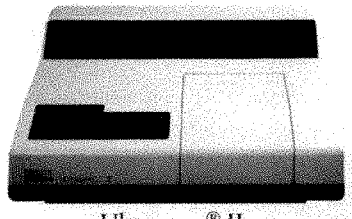
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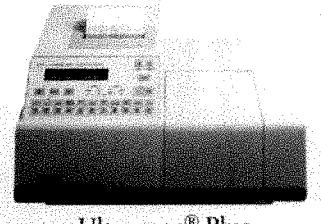
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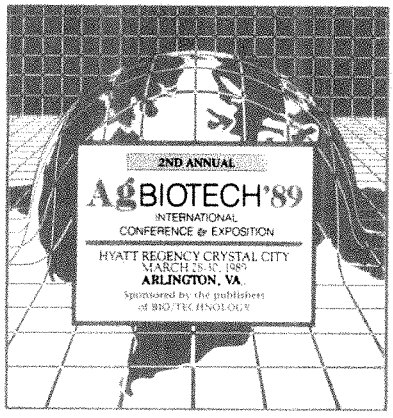

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Management Strategies
- International Strategic Alliances
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Wednesday, March 29

8:00 a.m. – 11:00 a.m.

Regulation
- Case Studies
- Testing For Registering Microbial Pesti

Chair: Edgar R. Butts/E.R. Butts International, Inc. and Robert B. Nicholas/Blum, Nash & Railsback

11:00 a.m. – 2:00 p.m.:

2:00 p.m. – 5:00 p.m.

Commercial Financing

Chair: G. Steven Burrill/Arthur Young

Thursday, March 30

8:00 a.m. – 11:00 a.m.

Patents
- Animal
- Plant

Chair: Kathleen Merrigan/U.S. Senate Committee on Agriculture, Nutrition and Forestry

11:00 a.m. – 2:00 p.m.:

1:30 p.m. – 5:00 p.m.

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ant Molecular Biology	Animals
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.
e Production of Transgenic ops Dicots - Cotton, Soybean, Oil Seed Rape, Walnut/Pine air: Harvey Bialy/BIO/TECHNOLOGY	Transgenic Animals Molecular Markets in The Genetic Improvement of Farm Animals Chair: Charles Arntzen/Texas A&M
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS	
2:00 p.m. – 5:00 p.m.	2:00 p.m. – 5:00 p.m.
e Production of Transgenic Crops Monocots - Rice, Maize, Asparagus air: Indra Vasil/University of rida - Gainsville	Transgenic Animals (continued) Chair: Charles Arntzen/Texas A&M

Wednesday, March 29

ant Molecular Biology	Animals
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.
otechnology, Plant Breeding d Crop Improvement air: Marc Van Montagu/Plant netic Systems	Animal Healthcare - ■ Vaccines ■ Diagnostics ■ Immune Stimulation Chair: Fred Brown/Wellcome Biotech
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS	
2:00 p.m. – 5:00 p.m.	2:00 p.m. – 5:00 p.m.
ntrolling Gene Expression air: Harvey Bialy/BIO/TECHNOLOGY	Animal Healthcare (continued) Chair: Fred Brown/Wellcome Biotech

Thursday, March 30

Perspectives	Biopesticides
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.
rogen Fixation air: Ethan Signer/MIT	Biopesticides ■ Bacillus thuringiensis ■ Endophytes ■ Baculorires Chair: Jerry Caulder/Mycogen
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS	
2:00 p.m. – 5:00 p.m.	2:00 p.m. – 5:00 p.m.
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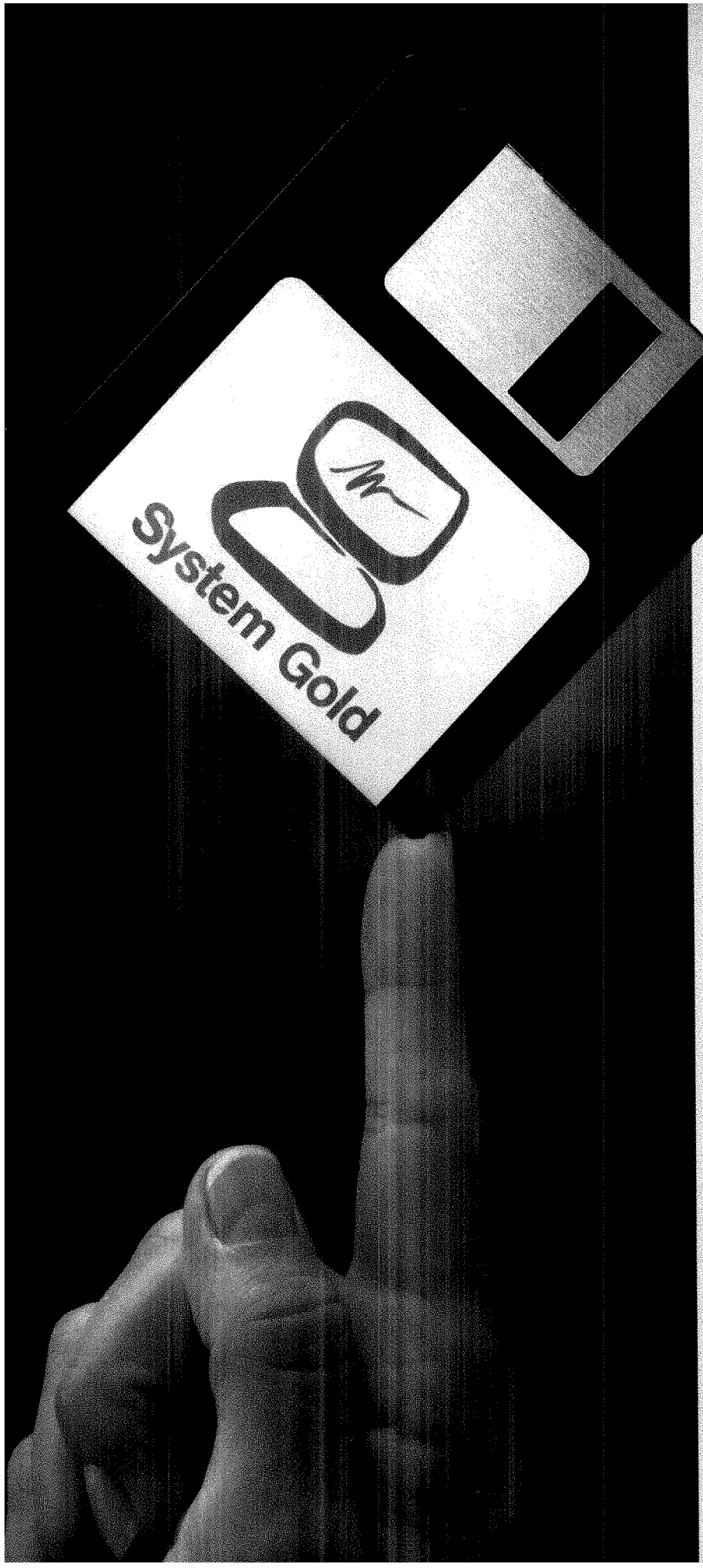
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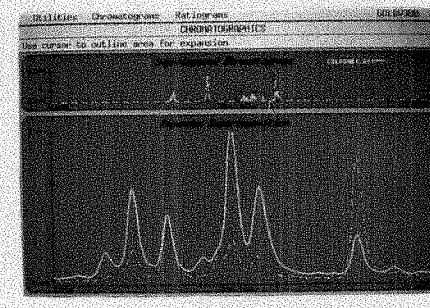
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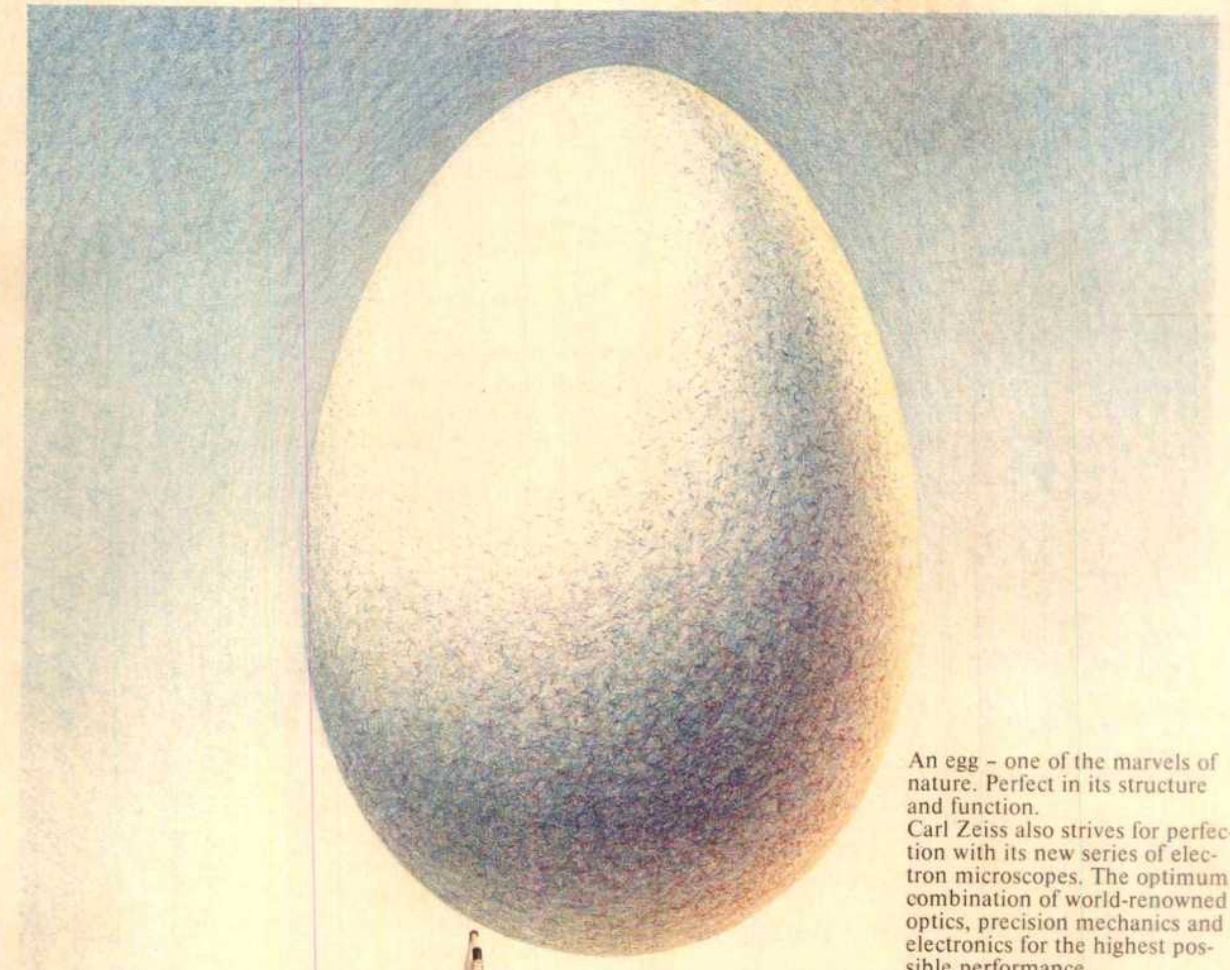
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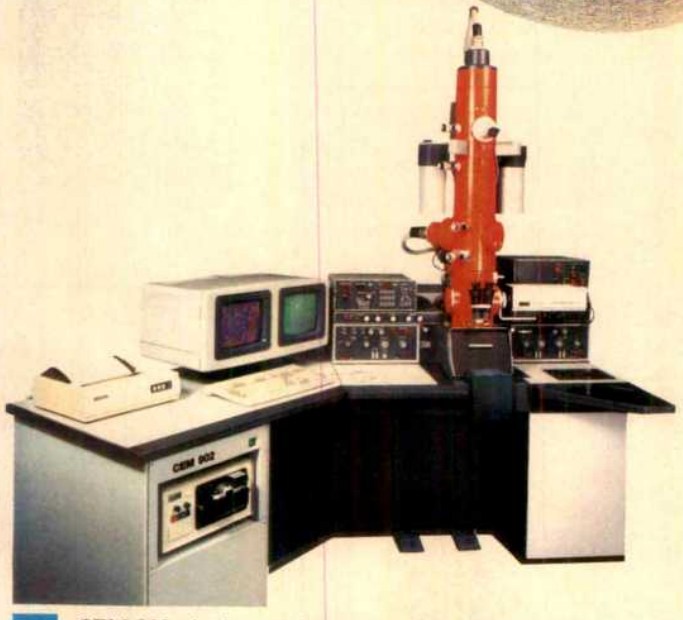
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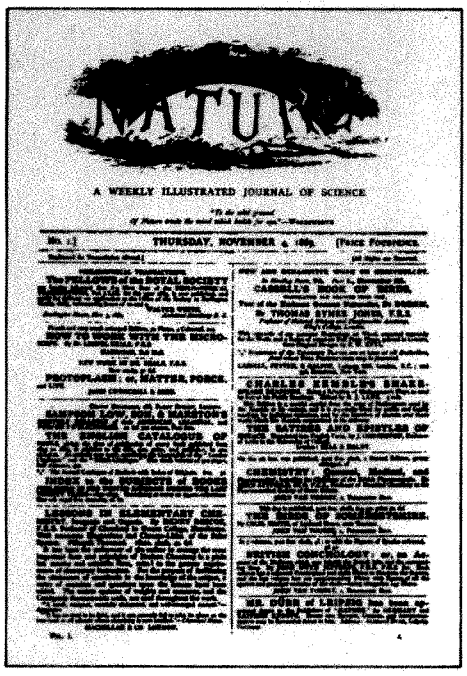
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nature

Making budgets in a straitjacket

The new US president has signalled how he would have spent money if there were more of it, and has singled out public education. The academic community should see that the message is amplified.

PRESIDENT George Bush's heart is in the right place, but his pockets are filled with lead. That is the first conclusion about the new US presidency to be drawn from the extraordinary occasion last week when the new incumbent, with beginner's bustle, rolled together the traditional State of the Union Address with the much-awaited explanation of how his budget will differ from that submitted by his predecessor to the US Congress just a few weeks ago (see *Nature* **337**, 104 & 200; 1989). Bush said all, or nearly all, the right things, but does not command the resources to make his wishes come true.

The new administration's difficulties have been plain for several months. To his credit, the new president did not hide from them last week. The formal constraint is so to manage planning for the next financial year (beginning on 1 October) that the statutory calculation in August of next year's deficit will come out at less than the $100,000 million allowed by the Gramm–Rudman rules.

The less calculable but more serious objective is to manage affairs so as not to disturb those elsewhere on whose willingness to continue lending to the United States depends the remarkable stability and growth of its economy. The new men at the White House have calculated that continued economic growth (an optimistic 4.5 per cent next year) will give them, in a year in which tax revenues increase above $1 million million for the first time, nearly $88,000 million extra to play with. No less than $64,000 million of that will be thrown into the expected spending gap, leaving a relatively small amount to meet extra inescapable costs. No wonder that the new president last week could make only token financial gestures towards the goals he has set himself for the four years ahead.

That does not mean that the goals are negligible. Under the rubric of US competitiveness, the Bush administration avows its support for science and technology (extending the tax credit on research and development spending by companies, affirming that the budget of the National Science Foundation must be twice that in 1988 by 1993 and even blessing the Superconducting Super Collider by name). Under the rubric of the "kinder and gentler society" which mocks the recollection of last year's bruising election campaign is a variety of environmental programmes that promise well (but will cost money only in future).

New programmes

The war on drugs is to be prosecuted with vigour, but again with real spending deferred. And the budget for next year includes a clutch of entirely novel spending programmes in support of public education in the United States which are to be judged by what they say about the president's intentions, not for what they will or even might accomplish.

While the US Congress may (and probably will) think differently, it will seem to many to be cruel that this brave talk should be heard when the US government is more constrained than ever by the lack of funds. Yet the truth is even more cruel than that. For the point seems to have been reached, in the brave history of the United States, when the future is constrained not simply by the gap between the US Treasury's spending and its disposable income, but by the gap between the ambitions of the United States as a whole and its disposable intellectual resources. For the state of public education in the United States, acknowledged for at least the past decade to be dreadful, should now be recognized as the most serious obstacle to the attainment of this and future new presidents' forgivably optimistic goals.

The circumstances are amply catalogued. The study of public education commissioned at the end of the Carter administration vividly drew attention to some of the more obvious problems — the difficulty of staffing engineering schools except by relying on graduate students from overseas, for example. By 1983, the Reagan administration's own contribution to the catalogue of neglect (called *A nation at risk*) declared that "a rising tide of mediocrity . . . threatens our very future". For a little while thereafter, several progressive states (which shoulder direct responsibility for education) made headway with schemes for improving the condition of elementary and secondary schooling, many of which persist. Yet the signs are that public education in the United States continues to deteriorate. Two years ago, a committee of the Carnegie Corporation under Dr Lewis Branscombe (then at IBM, now at Harvard) made the same point about the dependence of economic prosperity on the quality of young people's education, and chilled its audience with unflattering comparisons between the performance of children from the United States and elsewhere (in *A nation prepared*).

Promise of success

Since then, other more sombre truths have come to light — for example, the failure of 76 per cent of black students to graduate within six years from the universities they had begun to attend in 1980. It is no wonder that President Bush, like most candidates in last year's several election campaigns, is vowing again to fulfil the traditional promise of the United States that all newcomers, native-born or otherwise, will have an equal crack at success.

How well has he begun? The spattering of new programmes offered last week will not by themselves make much difference either way. Awarding prizes to successful schools and teachers may marginally encourage the others, while the plan to let each member of the Congress nominate a recipient of a $10,000 scholarship in science or mathematics will at least set senators and congressmen wondering about the causes that have thrust this patronage upon them. What matters more is that the new administration has declared its readiness to help stop the rot. Will the intellectual community in the United States be willing to seize the opportunity thus presented?

On the face of things, there is little that can be done. Public education, after all, is the responsibility of state governments, while one of the more obvious impediments to reform — the desperate shortage of able teachers — seems intractable unless all teachers are paid more, which would cost fortunes that no longer are easily manufactured. In any case, in the United States as elsewhere, academics working outside the elementary and secondary sectors of education have tasks of their own with which to grapple. The competition for research grants is as fierce as ever, but the administrators are now even more determined that overhead costs should be recovered. Yet like the US economy, US higher education rests on the now-shaky founda-

tion of elementary and secondary education. The all-too realistic fear in the United States that the economic competition with Japan (and even Europe) will eventually be lost stems from the acknowledged poverty of public education. Should not universities and other institutions of higher education recognize that their survival will even more literally turn on the future health of elementary and secondary education?

But what can research and higher education accomplish in such foreign fields? If it were that the problems of public education in the United States could be solved by spending money, it would probably be a better investment in the future to scrap the Superconducting Super Collider, or the extra shuttle flights, or even the National Science Foundation's doubled budget, for the sake of the improvement that those funds might buy. But money by itself is not sufficient. The more urgent needs are for a better understanding of how schools function well, for a school curriculum which is at once stimulating and improving, for a general appreciation of the particular importance of the most elementary education and for plain speaking about the seriousness of the difficulties created by the continuing impoverishment of the school system. Who but those in the academic community can do all these things? And how can they be neglected when it is as plain as at present in the United States that sufficient attention paid to public education would solve many of the other problems preoccupying the new president? □

Fraud on the agenda

The US Institute of Medicine recommends how malpractice in research should be controlled.

LAST week's report (see page 588) from the Institute of Medicine in the United States is one of the first by an academy or society representing a substantial part of the research community anywhere to face up to the problems of misconduct in the practice of research. That is not nearly as reprehensible as it may seem. Although the past decade has been peppered with lurid accounts of how apparently diligent researchers have pulled wool over the eyes of colleagues, either by the downright fabrication of data or by plagiarism, it is natural that leaders of the research profession should have been slow to respond.

Something akin to charity first prompts the belief that isolated scandals have been made conspicuous by contrast with the general practice, which is honourable. Then follows the proper fear that formal inquiry will engender the general opinion that research is shot through with malpractice. In the circumstances, the Institute of Medicine has been courageous in breaking new ground with its investigation. It is forgivable that its conclusions should be as tentative as they are.

The underlying difficulty is, of course, familiar — that there is no way of telling how common malpractice may be. Banks may hope to fix upper limits on the extent of embezzlement by careful accounting practice, for their losses on this account must always be less than the funds they cannot account for, but there is no arithmetical yardstick by which the total volume of scientific discovery is conserved. So may not the publicized cases of outright misconduct be simply the tip of a much larger iceberg? While there is no means by which the magnitude of the problem can be assessed, that suspicion cannot be flatly denied. What that, in turn, implies is that the damage done to the reputation of the research profession is certain to be much greater than is just. Researchers should not be too indignant on that score; even the most honest stockbrokers have been given a rough time because of the cases of insider trading brought to light in the past few years. The research profession naturally has an interest in regaining its reputation. A little reflection should show that its paymasters, usually governments, should be similarly inclined. How is that to be accomplished? Last week's report points to several ways which are none the worse for being obvious.

Universities should be more diligent and vigorous in their investigation of complaints or even suspicions, as should be grant-making institutions, while journals and professional societies also have their parts to play. Inevitably, the report muses on the incentives for misconduct and, concluding that competition in research and the pressure to publish are in part responsible, asks that something be done to blunt the edges of these pressures. Nothing much is new; the novelty is that it has been said by a respected but independent research academy. But the outcome is not a recipe for what should now be done.

Yet everybody working in research must know the answer. First, research is a profession whose integrity is, by definition, of astounding integrity; if it were otherwise, so that all published data and calculations were suspect, the notion that people can see further than others, in Newton's simile, by standing on the shoulders of others (giants or otherwise) would be falsified, and the scientific enterprise would long since have ground to a halt.

The torrent of discovery in the past few decades is in itself a proof of the integrity of the fabric of science. That is what nervous politicians must be made to understand. But, second, precisely because of the general appreciation of the integrity of the research profession, it is natural that people whose inclinations are less honourable should be attracted to it. In research, the problem is further magnified by unavoidable competition and by the circumstance that people are not required to behave well towards each other. Then, third, because discovery is the process of coming to grips with what people do not understand, mistakes are unavoidable and frequent — and cannot always be unambiguously distinguished from malpractice.

These sociological observations, however trite, have a bearing on the problem the Institute of Medicine (for one) would like to solve — that of minimizing the incidence of malpractice. The first conclusion must be that the incidence can never be absolutely zero, and that attempts to make it so can only bring science to a halt. Second, the people best placed to tell when observations have been improperly carried out or described are the close colleagues and competitors of the author or authors. That no doubt explains why committees of inquiry in this field have had such a poor track record in the past few years.

University departments or their equivalents in other kinds of organizations are better placed to sense when things have gone awry, and to take remedial action quickly. That they have been less effective than might have been expected in the past few years reflects two uncomfortable tendencies — the growing importance of the principal investigator as an autonomous unit in the conduct of research, and the dominance of his relationship with an external source of funds over that with more immediate and discerning colleagues. While principal investigators as such have not been conspicuous among malpractitioners — more often, it has been the hands they hired — putting back that clock completely would be impracticable, and probably unwise. But there is a case for arranging that principal investigators should more often than at present be less dependent on an external grant-making agency, and more dependent on their colleagues locally. At worst, good researchers who are not good managers might be helped to deal perceptively with their assistants.

The Institute of Medicine's report follows most other lines of argument than this. There should be more guidelines, university officials with special responsibility for "the responsible conduct of research", a special office at the National Institutes of Health (and presumably other agencies) to investigate complaints and even courses of instruction for beginners in research. In reality, there is at least as strong a case for believing that grant-making agencies should be less, not more, immediately involved: they should rather confine themselves to making sure that grant-recipients' institutions arrange that their funds are spent responsibly. And most grant-making institutions in the United States are too close to Congress, and too remote from where the action is, to function effectively and quickly in the prevention of misconduct. The best they can hope to do is to function judicially after the event, which is a heavy-handed business. □

British research learns how to spend extra

■ New interdisciplinary centres under way
■ Three-year increases in science budget

London

ONE of the most recent innovations in the British pattern of research support, the interdisciplinary research centre (IRC) is to be the subject of a major review after the British government has expressed caution about the programme. The review, which is to begin this month and be complete by the summer, will cover the selection of topics on which the centres work, their management, links with industry and their evaluation.

This development was announced with the publication last week of the annual advice from the Advisory Board for the Research Councils (ABRC) to the Department of Education and Science about the allocation of the science budget for the years ahead. The occasion is especially important this year because £340 million will be added to the science budget over the next three years.

For next year, the extra money amounts to £107 million, bringing the total for 1989–90 to £825 million. Of the extra, a quarter will be absorbed by extra allocations already made to the British Geological Survey, the British Antarctic Survey, CERN (the European High-Energy Physics Laboratory), directed research on AIDS and the cost of further redundancies at the Agricultural and Food Research Council (AFRC).

In previous years, prior allocations have absorbed most of the extra funds available, but in the coming year there will be a significant amount left over to be divided among the research councils. Overall, the 1989–90 budget for civil science is 11 per cent greater than in the present year, which ABRC says will do much to improve morale. But ABRC also says that the allocations for the succeeding 2 years imply a reduction of 3 per cent in real terms, which it says will mean that research councils will have to cut back on the projects they support.

With the extra funds available for next year, the Natural Environment Research Council (NERC) will spend £2.5 million on the first phase of an IRC in population biology at Imperial College, London, and £5.5 million on a centre for oceanography at Southampton. It will expand work on the biogeochemical ocean flux study (£2.7 million) and on the North Sea survey, meant to lead to a model for predicting water quality.

The Science and Engineering Research Council (SERC), the largest of those suporting natural science research, will spend £7 million next year on founding four new IRCs, in optical and laser-related science and technology, high-performance materials, polymer science and technology and process simulation, integration and control. It will also start a new programme in materials and biotechnology with £5 million of its extra allocation. SERC also has an extra £6 million for research grants, £9 million for university equipment and £0.4 million for its Cray computer facility.

The Medical Research Council (MRC) can now go ahead with an IRC in cell biology (£2 million) at Kings and University Colleges, London, in protein engineering (£2 million) at the University of Cambridge and the MRC Laboratory for Molecular Biology. It also has an extra £2.6 million for AIDS research and £2.3 million to set up an information and resource centre for mapping the human genome together with a related programme for research.

A further £2 million will allow the council to make a start on a national centre for clinical research at the Royal Postgraduate School at Hammersmith. There will again be more to spend on research grants (£1.5 million), studentships (£0.2 million) and equipment (£2.9 million).

The chief new projects of AFRC are an IRC in transgenic animal biology at the University of Edinburgh (£1.5 million) and new programme of research in plant molecular biology (£3.5 million), but the council will also take the lead in the government's new programme of research on agriculture and the environment (£1.5 million). The cost of restructuring the Institute for Animal Health is £3 million.

The delay in the announcement of this year's allocation of the science budget has fuelled speculation of a dispute between the prime minister, Mrs Margaret Thatcher, and the Department of Education and Science. The Labour party's education and science spokesman, Mr Jack Straw, says that the prime minister is unhappy with the department's adminstration of the councils, and that she wishes to move ABRC from beneath its umbrella.

But others suspect her of having intervened to contain spending on the IRCs, having advocated support for indivdual researchers of talent working singly or in small groups. But both the department and the Prime Minister's office deny there has been a dispute. The future of ABRC is itself being reviewed by the department; a report is due in the summer.

Christine McGourty

The UK science budget agreed for 1989–90 and planning figures for 1990–91 and 1991–92

Additions	1989–90 (£ million)	1990–91 (£ million)	1991–92 (£ million)
'Earmarked additions'	27	33	33
Research council restructuring	14	12	11
Interdisciplinary research centres	16	21	14
'Directed' programmes	23	29	34
Research grants	10	13	14
Manpower	3	5	8
Equipment	12	—	—
Other	3	4	2
Total	108	117	116
Final budget	825.6	837.6	855.8

Ozone limitations

Paris

FRENCH industry is taking steps to limit the use of products containing chlorofluorocarbons and halons. A series of conventions signed last week by representatives of government and industry are intended to reduce by 90 per cent the use of these propellants in aerosols by 1991 — two years before the target date set by the Montreal Protocol.

Beginning in 1991, aerosol cans containing chlorofluorocarbons will have to carry a written warning declaring that their use could affect the ozone layer. Manufacturers of refrigeration and air-conditioning appliances will encourage the recovery of coolants and will take measures to prevent leakage if appliances are dumped.

From the beginning of next year, manufacturers will also attach a warning to new appliances, encouraging users to collect the coolant for recycling. This will require the redesign of cooling circuits to include a drainage reservoir, as well as establishing public facilities for recovery of used coolant.

The newly signed conventions will also limit the use of fire-extinguishers containing halons. Atochem, the only French manufacturer of chlorofluorocarbons has agreed to make alternative compounds available to users.

Peter Coles

NIH change procedures for monitoring scientific misconduct

Washington

STRUCTURAL changes are on the way in the procedures used by the National Institutes of Health (NIH) to monitor scientific misconduct. The impetus comes from a report released this week by the Institute of Medicine* (IOM) urging NIH to establish a new office to promote responsible research practices.

Although recent investigations have focused attention on misconduct in science, the IOM report looks instead at the broader question of what are appropriate standards for the conduct of research. It arises out of discussions on this issue between IOM and NIH held in 1985; a 17-member committee was appointed to write a report in 1987.

For the most part, the culture of science by its nature encourages proper conduct, says Arthur Rubenstein, chairman of medicine at the University of Chicago and chairman of the IOM committee. Tight budgets have put a variety of pressures on researchers, says Rubenstein. As well as encouraging a high rate of publication to substantiate high productivity, competition for scarce resources can tempt researchers to cut corners.

Rubenstein also cites the increasingly large size of laboratories, which makes it difficult for laboratory heads to fulfil their traditional role of monitoring the activities of their junior staff.

To combat these pressures, the IOM report suggests making explicit proper professional standards for research. Universities should set standards of conduct, provide formal instruction in research practices and create administrative positions carrying responsibility for conduct. Journal editors and professional societies are also asked to demand such behaviour from their contributors and members.

The IOM report explicitly rejects the suggestion that random data audits should be carried out in basic research. Rubenstein explains that the nature of basic research makes it nearly impossible to trace all the steps from data collection to tabulation and presentation, steps that can change as circumstances and new discoveries dictate. Such audits are more appropriate to clinical and protocol based research, where they are already used.

Meanwhile, NIH is urged not only to establish an office to encourage proper conduct, but to require by 1992 all institutions in receipt of grants to show that they have addressed the issue.

The report also suggests that NIH should limit the number of publications considered as part of a grant application to ease the temptation to produce inappropriately voluminous *curricula vitae*.

Rubenstein says the research community is now more open to suggestions that questions of conduct should be tackled, but he expects that there will at first be resistance to the proposal that there should be special panels for addressing questions of proper conduct. He believes the initial resistance will subside when researchers see that panels can improve the research climate.

NIH deputy director William Raub says the institutes, together with the Department of Health and Human Services, are already discussing the creation of an office of scientific integrity that could fill the role recommended by the IOM report, as well as investigating charges of misconduct. Raub says a crucial issue to be settled is where the office should be placed in the federal bureaucracy. The four possibilities being discussed are the Office of the NIH Director, the Office of the Assistant Secretary for Health, the Office of the Secretary of Health and Human Services (HHS) and the Office of the Inspector General at HHS.

The last of these options is the least favoured because the inspector-general's staff lacks scientific expertise. A final decision is bound to wait on a resolution of the political question when the position of secretary of HHS is filled. President Bush's candidate is still waiting confirmation by the US Senate.

Although the IOM report focuses on biomedical research, it recommends that the National Academy of Sciences look into the relevance of its findings for other scientific disciplines. **Joseph Palca**

**The Responsible Conduct of Research in the Health Sciences.* National Academy Press, Washington, DC, 1989.

US congress scuttles federal agency salary increases

Washington

THE US Congress voted last week to scuttle a 50 per cent pay increase it had been offered, and thereby perpetuated a problem with which federal agencies employing highly qualified professional staff have been struggling for some time. The National Institutes of Health (NIH) is among the most outspoken of the agencies affected.

NIH claim that their ability to recruit and retain able researchers has long been compromised by federal salary scales well below those applying at universities and in industry. The federal pay rise, which at first seemed certain to pass, would have applied to senior researchers and administrators at NIH and would, according to officials, have solved an old problem.

The proferred salary increase, recommended by an independent commission reporting every four years and endorsed by former president Ronald Reagan, would have taken effect automatically at 12:01 am on 8 February unless both the House of Representatives and the Senate had passed resolutions to reject it. The Senate did vote against the rise, but the House of Representatives had at first planned to let the deadline pass without taking action. But a huge public outcry against the proposal seems to have forced a vote — in the circumstances, inevitably negative.

After the vote, Senator Ted Stevens (Republican, Alaska) lambasted Congress for being unwilling to take the political heat for approving its own pay raise, a move that "penalizes other portions of the federal government", in particular NIH. Unlike most government laboratories, which are run by contractors who are not bound by federal pay scales, NIH researchers are federal employees.

NIH Deputy Director William Raub says that in the past ten years the number of NIH employees at the senior executive level has decreased by 28 per cent to the present 174 people. During the same period, NIH have not been able to recruit a single person from outside the institutes. Some of the decrease is due to retirement but Raub maintains many scientists have left for higher paying positions outside government. As an example, he says that senior executive service physicians receive on average $89,000 a year, only half the average supplemented salary paid to chairmen of clinical science departments at universities.

An independent committee of the Institute of Medicine, in a report issued last year (*Nature* **336**, 701; 1988), agreed that there is a serious problem at the senior level and suggested two remedies — the establishment of an NIH foundation to finance chairs for distinguished scientists, whose salaries would then be independent of government pay scales, and the creation of a personnel demonstration project within NIH that would allow more flexible hiring and remuneration.

Raub says the committee's report is "right on the mark". Now that Congress has rejected the pay rise, NIH are looking at the report's suggestions in more detail, but cannot hope to follow them quickly because that would require that a reluctantly self-denying Congress should pass new legislation to give others the benefits of which it has been robbed.

David Swinbanks

India's troubled space plans receive unexpected French boost

New Delhi & Paris

FRANCE last week signed bilateral agreements with India in the life sciences and agreed to help with India's troubled rocket programme and to strengthen nuclear ties between the two countries. The agreement on nuclear power would permit the French company Framatome to build two 900-MW light-water nuclear reactors in India provided that India agreed to use them for peaceful purposes only.

In the space arena, France has offered assistance for India's launch vehicle programme, which has been lagging after two successive mishaps. Hubert Curien, France's minister for research and technology, says immediate assistance can be given on the technology for clustering rocket engines, but a request for help with cryogenic motors may be delayed because of its commercial implications.

Curien says France could still provide this technology in consultation with its European partners if India agreed not to put the technology to military use and avoid any future competition with Arianespace in the international launch business.

According to the terms of the life sciences agreement, a joint venture company will produce six different vaccines using the technology supplied by Institut Marieux, which will hold 25 per cent of the company's share capital. The vaccine plant, to be built at Gurgaon near New Delhi, will start commercial production in 1991.

Defence cooperation was not on Mitterrand's agenda because the subject had already been discussed during the visit of the French defence minister in December 1988. France is said to have offered a $2,000 million package for collaboration that includes a contract for the design of India's third aircraft carrier, the supply of an advanced jet trainer, air-to-air missiles and anti-tank helicopters. Avions Marcel Dassault of France has been hired as a design consultant for the light combat aircraft India is building.

The French cultural festival in India that prompted Mitterrand's visit had a marked edge of high technology, as was evident from the inaugural show — a spectacular extravaganza in Bombay's Chowpathy Bay. Thousands of Indians, in the company of Mitterrand and Prime Minister Rajiv Gandhi, watched the bay reverberate to amplified music, with giant water screens providing the backdrop for eerie images — fusions of art and science — drawn by computer-controlled laser beams reflected from huge mirrors atop barges amid a massive display of pyrotechnics that set the night on fire. According to a French diplomat, the effort was worth it if it had helped to impress on Indians that France can offer something more than wine and cheese.

K.S. Jayaraman & Peter Coles

First report on US AIDS behaviour

Washington

THE first report* solely to address the behavioural aspects of the AIDS epidemic was released last week by a committee of social scientists organized by the US National Academy of Sciences. The committee decried the dearth of information available on sexual behaviour and intravenous drug abuse, and called for more studies to find viable ways of changing people's behaviour to lower their risk of infection with HIV, the virus causing AIDS.

The committee's report is the fourth major analysis of the AIDS problem to be conducted in the United States. The Academy and the Institute of Medicine teamed up in 1986 and again last year to offer their assessment of how to counter the epidemic, and the presidential commission on the HIV epidemic issued a laundry list of about 575 specific recommendations last year. Although some of the recommendations in these reports have been implemented, more controversial steps such as anti-discrimination legislation and random blood testing for HIV antibodies have been snarled in political battles.

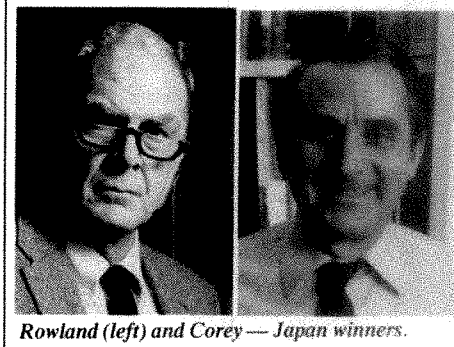
The Academy committee recommends more systematic studies of human sexual practices and drug abuse patterns. Committee chairman Lincoln E. Moses from Standford University says "AIDS is a social disease", and that not enough effort has so far been put into studying the behaviours that put people at risk and learning how best to motivate them to change. The committee also recommended anonymous blood testing of all newborns.

Marshall H. Becker, from the School of Public Health of the University of Michigan, says the "Just Say No" approach favoured by the Reagan Administration is not working, and that more practical approaches for reducing risk must be used. Among the committee's recommendations for encouraging behaviour changes are expanded sex education for teenagers, condom advertising on television, and free distribution of needles to drug addicts until they enter treatment programmes. Carol Ezzell

**AIDS: Sexual Behaviour and Intravenous Drug Use*, available from the National Academy Press, Washington, DC, 1989.

Japan prize awards

THIS year's Japan prize has been awarded to two US chemists working in different fields. The ¥50 million ($400,000) awards will go to F. Sherwood Rowland, an atmospheric chemist at the University of California at Irvine, and to Elias James Corey, an organic chemist at Harvard University. They will be presented on 12 April in Tokyo.



Rowland (left) and Corey — Japan winners.

Rowland is recognized for establishing that chlorofluorocarbons (CFCs) can destroy ozone in the stratosphere (see *Nature* **249**, 810; 1974). He predicted that if CFCs were to continue to be released into the atmosphere at 1974 rates, there would be a 7–13 per cent depletion of total ozone. Corey's award is for his contributions to the synthesis of prostaglandins.

The Japan prize is awarded each year in two predetermined categories. This year's categories were medicinal science and environmental science and technology. Next year's will be for the technology of integration and for Earth sciences. J.P.

Peking man safe?

THE municipal government of Beijing (Peking) has introduced regulations to protect the site, now in the suburbs of the city, where the remains of Peking man were found. Under the new regulations all activities are prohibited that may destroy the land-form, terrain and ecological environment, including mining, tree-felling, hunting and the construction of factories that could be a source of pollution. Illegal excavation or selling or buying fossils and remains are also prohibited. V.R.

EMBL *déjà vu*

LENNART Philipson, director of the European Molecular Biology Laboratory in Heidelberg, will continue in his post until 1995 rather than leave next year as originally intended.

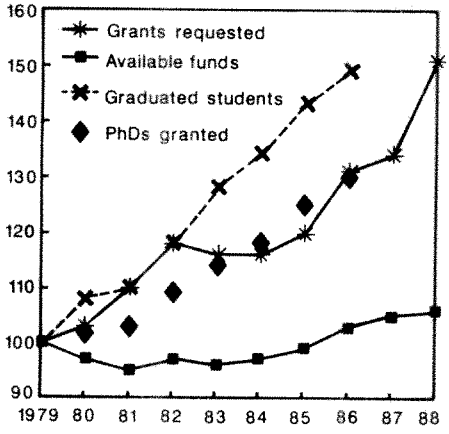
Faced with the withdrawal of the only candidate to replace Philipson (see *Nature* 337, 397; 2 February 1989), the laboratory's council last week decided to extend Philipson's stay. His original appointment was from 1982 to 1987, which was then extended for only three years. As a condition of continuing in post beyond 1990, Philipson successfully insisted on a five-year reappointment. P.N.

West German agency launches early bid for more funds

Bonn

THE Deutsche Forschungsgemeinschaft (DFG) has scored an early success in its campaign for an increased budget. West German Education Minister Jürgen Möllemann (Free Democrat) announced last Friday that he will campaign for a 5 per cent annual increase for DFG, the chief source of research grants in West Germany, in each of the next 5 years.

The proposed increase is by no means a certainty, since all eleven *Länder* (states) must first approve it. On the federal level, Möllemann must secure the support of the rest of Chancellor Helmut Kohl's cabinet. Three-fifths of DFG's support comes from the federal government and the rest from the *Länder*. Asked about the chances of success, Möllemann said that an annual increase of 5 per cent is "realistic and achievable". The agency's budget this year is DM1,188, but the 1990 budget will not be finally approved until December.



Grants requested and funds available from the DFG to 1988. The 1979 figures are taken as 100; no allowance is made for inflation.

DFG President Hubert Markl had sounded an alarm earlier last Friday when he declared that basic research support had "dramatically worsened" in 1988. He predicted grave consequences if DFG did not receive a 6.5 per cent increase in 1990.

The nub of Markl's case is that, in 1988, DFG had to reject the highest percentage of grant applications in its history: only 48.4 per cent of individual applications were funded. And for the first time, fewer than half of the *Schwerpunktprogrammen* (priority programmes) applied for could be supported (see *Nature* **336**, 510; 1988).

Part of the difficulty is that research is booming at West German universities, so that there are more applications for grants than ever. Markl expects even more competition for DFG funds in the future: 40 applications have already arrived for *Sonderforschungsbereichen* (special collaborative programmes), half of them planned to begin before 1990.

There are also more students than ever crowding West German universities, which Markl says will be looking to DFG to support graduate and postdoctoral studies for the next decade. Markl says that if these people are forced out for lack of funds, their potential will be lost.

One consequence of the high rejection rate is the disaffection of DFG's (unpaid) referees, who Markl says are "fed up" with reviewing good proposals which are not supported. But he resists the suggestion that greater competition might enhance the quality of West German research: that point was reached years ago, he says, so that the applications now being turned down are of the highest quality. Markl is concerned that Wes Germany will be "left behind" if it doe not increase research support. He point to the planned 10 per cent increase of th budget of the US National Science Found ation and the similar increase for the cor responding body in Japan.

DFG received only a meagre increase i 1988, despite Markl's impassioned appea in June, which is why he has felt impelle to act earlier this year, writing to Kohl an the heads of the *Länder* in January. H was visibly pleased by Möllemann' announcement last Friday.

The proposed increase is likely to b discussed at the next conference of th heads of the *Länder* on 10 March. Well-of *Länder* such as Baden-Württemberg an Bavaria are expected to take up DFG' case, while Möllemann's announcemen will put political pressure on the others.

Steven Dickma

Soviet psychiatrists at odds on eve of visit by United States

London

THE campaign by Soviet psychiatrists to rejoin the World Psychiatric Association may be impeded by an open letter to the Soviet Academy of Medical Sciences by Dr Viktor Gingilis, head of the genetics laboratory of the Brain Research Institute at the All-Union Research Centre for Mental Health. The letter is a direct attack on the director of the institute at which Gingilis works, Dr Marat E. Vartanyan.

As director of the Centre for Mental Health (since December 1987), Vartanyan is in a position to influence the practice of psychiatry in the Soviet Union. The letter, first circulated at the end of last November, comes on the eve of the beginning next week of a 2-week tour of Soviet psychiatric hospitals by a group of 20 US psychiatrists and other experts.

The charges levelled against Vartanyan, who is known in the West for his categorical denials that sane but politically suspect persons were ever incarcerated in Soviet psychiatric hospitals, include many of a personal nature. But Gingilis's general complaint is that Vartanyan is seeking to shift onto others his own burden of responsibility for a practice in which he was "most active".

Reached by telephone at the institute, Gingilis acknowledged authorship of the letter, but said that he himself had not sent it abroad or to the media. Asked whether, in that case, he would prefer *Nature* not to report it, he replied "That's your affair".

Vartanyan was not available for comment, being about to leave on a business trip. His assistant, Dr Georgii Morozov, said, however, that Vartanyan knew all about the letter, and thought it a matter of little importance. "If Dr Vartanyan could speak to you, he would explain itall", Morozov said. An arrangement tha Vartanyan would call *Nature* back at an agreed time failed to materialize.

Gingilis says that Vartanyan's election to his present post (necessary under the new democratization) was rigged, and went against the wishes of the majority of the centre's staff. His letter goes on to suggest that Vartanyan and his wife, D. D Orlovskaya (who is at the same institute) have risen in academic distinction on the strength of the work of others, many of whom have subsequently been hounded into resignation or emigration. The letter says "I am the real author of almost all the publications and research initiatives attributed to M.E. Vartanyan in the field of medical genetics".

Gingilis claims that his own work, together with that of I. V. Shakhmatova-Pavlova, was misappropriated by Vartanyan to support his appointment as corresponding member of the Soviet Academy of Medical Sciences. He says that Vartanyan's personal power base has remained secure because few have been prepared to speak up in public, but "after twenty years of serf-like dependency on Vartanyan", he has decided to speak out "with all responsibility and with a clear understanding of the possible consequences".

It remains to be seen what will be the effect of this letter on next week's visit by the US group. The Soviet authorities maintain that all dissidents have either now been released, or are being processed for release, from psychiatric hospitals. The visitors will be able to examine patients whom they choose and to visit at short notice psychiatric hospitals which they select.

Vera Rich

Too high radon risks not helped by "penny-pinching" UK government

London

BRITISH safety limits for the concentration of radon gas in houses are too high and government proposals for financial help to those at risk are "penny-pinching and restrictive", according to the Institution of Environmental Health Officers (IEHO). The institution is the professional organization of environmental safety officers employed by local governments. Its criticisms of government policy appear in a report published two weeks ago.

The key recommendation is that the radon dose at which remedial action is considered to be necessary should be



halved, from 400 to 200 becquerels per m^3. This concentration was fixed two years ago by the National Radiological Protection Board (NRPB), when it was estimated that the lifetime risk of lung cancer after chronic exposure to 400 Bq per m^3 would be between 2.0 and 2.5 per cent.

The NRPB now estimates the lifetime risk to be 5 per cent, and the environmental health officers say that, as a result, the action level should be halved. The NRPB is now reviewing its safety levels. But Michael O'Riordan of the board says the level will not automatically be halved: the cost of taking remedial action and the available technology will be taken into consideration before a decision is made.

Only in the United States is the action level lower than in Britain: the Environmental Protection Agency (EPA) recommends an action level of 150 Bq per m^3. The agency would like this to be reduced by a factor of 10, at which point the radon concentrations indoors would be equivalent to those outdoors. But in practice it may only be possible to halve the present action level, says a spokesman. About 8 million homes, 10 per cent of the US total, are estimated to have concentrations of radon above the action level. The most common way of tackling the problem is to "poke a hole in the floor of your basement, put a pipe in, run it through the ceiling and put a fan on the top", according to an EPA spokesman. This can cost between $200 and $1,500. The EPA is carrying out a survey to find out how many take the necessary action.

In the European Communities, no country other than Britain has any safety levels for radon concentration in the home. But the European Commission is considering recommending that member countries adopt the British standards. Sweden and Finland, the only other European countries with safety levels, set the action level at 800 Bq per m^3.

The IEHO report puts the number of houses in Britain with radon levels above the present action level at 50,000, which is also twice the number estimated by NRPB. As well as the halving of the action level for which it asks, the institution says that the "priority action" concentration should be reduced from 1,000 to 800 Bq per m^3 and that the "investigation" concentration should be reduced from 200 to 100 Bq per m^3.

The report is especially critical of a government document giving guidance to local authorities on home improvement grants to those potentially at risk. The government expects most owners to bear the cost of remedial action themselves, but Martin Courtis, the report's author, says that if house owners are not offered a grant of at least 75 per cent of the cost of remedial work, there is little, if any, prospect that the radon problem in Britain will be tackled. **Christine McGourty**

Computer microcode instructions judged within copyright

Berkeley

A Californian court last week declared that microcode instructions on microprocessor chips can be protected by copyright. The decision, expected to be a landmark for microprocessor manufacturers, is likely to deter outright copying of microprocessor design.

Although computer programs were added to the US Copyright Act by Congress in 1980, the status of microcode — the set of instructions determining how a processor's components will perform a specific function — was unclear. As with any other program, a variety of instruction sets could be devised to achieve the same goal. Last week's ruling puts microcode "squarely within the definition of 'computer program'", and therefore subject to copyright protection.

The ruling also ends a 7-year dispute between Intel Corporation, of California, and NEC Ltd, of Japan. Intel claimed that NEC's 'V-series' of microprocessors infringe Intel's copyright of its 8086 and 8088 chips, used in the first generation of IBM personal computers. Although the court upheld Intel's right to copyright the microcode, it nonetheless absolved NEC of having violated that copyright, allowing both sides to claim victory in the case.

Intel and NEC entered into a general patent cross-licensing agreement in 1976 giving each access to the other's patents. The copyright dispute arose after Intel introduced the 8086 and 8088 microprocessors in 1978, and NEC began producing identical copies of the chips under the cross-licensing agreement. Intel objected, arguing that the patent-sharing allowed NEC to produce a chip of similar function, but not to use the copyrighted microcode instructions contained in the chip. The matter was temporarily settled in 1983, when Intel licensed NEC to make the chips. Shortly afterwards, NEC developed its own 'V-series' of comparable microprocessors.

The first trial of the lawsuit was discontinued in 1986, when NEC objected that the judge owned a small amount of Intel stock. The judgement in the second trial, announced last week, declared that microcode can be protected by copyright, but found that NEC's microcode is sufficiently different from Intel's not to be a violation. Intel was also found to have forfeited its copyright by not requiring licensees to place notice of the copyright on their chips. NEC now has 11 per cent of the $7,000 million microprocessor market, compared with Intel's 26 per cent share, but NEC officials predict that the court's vindication of its practice will improve its sales.

But Intel is also claiming victory, as the decision is likely to discourage others from copying its microprocessor designs. The loss on the 8086 and 8088 chips is financially unimportant, say company officials, as the 10-year-old chips have been replaced in personal computers by second- and third-generation microprocessors. The 8086 and 8088 chips now account for only 1 per cent of total Intel sales, says company representative Pam Pollace. She also says that the more complex microcode of the second- and third-generation 80286 and 80386 microprocessors will be much more difficult to approximate except by outright copying, which would clearly infringe Intel's copyright. **Marcia Barinaga**

Oil-spill danger to nesting birds

Berkeley

A US oil cleanup crew last week reached the Antarctic Peninsula and stemmed the flow of diesel fuel from the wrecked Argentine supply ship *Bahia Paraiso*, which ran aground on 28 January near the US Palmer Base. The ship had already spilled two-thirds of its 250,000-gallon fuel supply. While the US team skimmed most of the recoverable fuel from the surrounding waters, an Argentine salvage crew prepared to remove the remaining 75,000 gallons of fuel remaining on the ship.

The biological consequences of the accident are now being evaluated. Unrecovered fuel has collected in shallow coves and inlets, where it has killed intertidal limpets, sponges and kelp, says Bernhard Lettau, National Science Foundation (NSF) programme manager for Polar Ocean Sciences, who recently arrived at the site. Lettau says the bottom-dwelling community may escape serious harm, as the water is relatively clear and free from particulate matter that could carry the fuel to the bottom.

But oil slicks less than 1 mm thick cannot be removed by skimming, so that a diesel sheen remains on much of the water in the area, according to Thomas Forhan, an NSF project manager at the site. He says that all traces of the volatile fuel should be gone within 10 days to a month, barring further leaks.

Forhan says that the ship, which is on its side in 40 feet of water, is unlikely to break up soon and that the Argentine salvage operation may take 3 to 4 weeks to recover the remaining fuel. He notes that the recovery entails the risk of further spills.

The effects of the spill on the area's krill population are unclear, according to Lettau. Deep-water animals have been less badly affected than shallow-water ones. But the spill has had a devastating impact on bird populations nesting near Palmer Base. Lettau says the breeding success of the South Polar skuas in the area "will be zero". Skuas lay two eggs in each nest, and usually only one chick survives to fledge.

But out of 53 nests inspected, only 6 had a living chick last week, and Lettau expects none of these to survive. He is unsure of the cause of death, but speculates that the chicks may be getting toxic doses of fuel in the food brought by their parents.

Lettau expects that the toll will be greatest among young birds, including penguin chicks, which fledge and take to the water in early February. Researchers at the base have begun banding chicks that have not yet left their rookeries, so as to trace their eventual fate. **Marcia Barinaga**

Japan grapples with definition of death by brain death

Tokyo

THE twenty-year controversy over the definition of brain death has entered a new phase. Last week, the Japanese Diet (parliament) was presented with new legislation that will eventually decide when the absence of electrical activity in the brain (brain death) can be considered to define the death of a person. At stake is whether surgeons may legally perform life-saving heart and liver transplant operations — both of which require the donation of living organs from brain-dead people.

The legislation, which seems certain to pass, would establish a special investigatory panel on brain death and organ transplants. The panel is modelled on the US Presidential Commission on Ethical Problems in Medicine and Biomedical and Behavioral Research which, in 1981, accepted that brain death means death. But the new panel will take two to three years to reach its conclusions, which may be far too long for Japan's surgeons.

Already, a Niigata Prefecture hospital group has twice risked prosecution by performing a kidney transplant operation with an organ taken from a brain-dead patient. The first operation, last May, put the group in the odious position of having to face a legal suit for murder. Despite the risk of legal action, several other groups are eager to claim the prize of Japan's second heart transplant. The first, carried out in 1968, brought legal action and vilification for the surgeon concerned.

But now, after twenty years, a new force is propelling Japan towards acceptance of brain death. To Tokyo's embarrassment, Japanese are starting to travel abroad for transplant operations they cannot get at home. Last week, at almost the same time as the Ministry of Health and Welfare announced a new Tokyo research centre to help developing nations learn from Japan's 'advanced medical treatment' system, a fact-finding mission reported that eight Japanese patients were waiting in the Philippines for transplant operations. The mission, led by Representative Taro Nakayama (Liberal Democrat, Osaka), who has been active in promoting the new Diet legislation, also visited Australia, where it found that 22 Japanese had either received, or were waiting for, liver transplants.

With transplant organs in short supply everywhere in the world, Japanese patients in the hospitals of developing nations would seem especially incongruous. The Philippines is already considering legislative action to control visits by foreign patients.

Early last year, it seemed that Japan's brain-death problem was about to be resolved when the Japan Medical Association's Bioethics Council released a report urging that, subject to strict conditions, brain death should be accepted as a definition of death. Surgical groups at several hospitals immediately began to file applications with their own hospital ethical boards for permission to perform transplants.

But the momentum began to wane when the Japan Federation of Lawyers Associations announced its opposition to the redefinition of death as brain death until a full social consensus had been reached. Subsequently, in May, the Japanese Society of Psychiatry and Neurology decided to come out against the redefinition.

At nearly the same time, a University of Tokyo patients' rights group instigated legal action for murder against those responsible for the Niigata Hospital kidney transplant. The group's view is that patients' rights are not adequately protected from surgeons wishing to carry out transplant operations. Although that view finds few supporters among physicians, public criticism inhibited further transplants from brain-dead patients in 1988.

The Niigata group censured last year has already performed one kidney transplant from a brain-dead patient this year. According to relatives, the patient had expressed a wish to donate his organs in the event of his death.

Writers of Nihonjinron, a genre that explores supposedly unique characteristics of Japanese culture, have often asserted that the aversion to acceptance of brain death and transplant operations is related to a Japanese view that body and soul are inseparable, or to confucian concepts that the body is a gift from one's parents that cannot be given away. But the Niigata man who left instructions for his organs to be donated is reported to have been much influenced by a television progamme on brain death.

The debate over definition of death has mostly subsided in the United States and Europe, with whole brain death being the commonly accepted criterion. Some have argued for extending the definition to include loss of higher brain function, but this has not gained acceptance. In most countries, solid organs may be retrieved from cadavers only with prior permission from the victim or the permission of relatives after death. But in France there is 'presumed consent', so that organs may be taken if needed over the objections of relatives unless the victim had previously made an express wish to the contrary.

Alun Anderson

Continuing discontent among Soviet scientists over elections

Moscow

CONTINUING discontent with the Soviet Academy of Science's election of nominees to the Supreme Soviet took an extraordinary turn on 2 February — a public meeting of protest outside the academy's main building in Moscow which lasted for an hour and was attended by 3,000 people.

Although public meetings are becoming a routine part of the process of democratization, the meeting outside the academy was without precedent. It is only fair to say that the academy did nothing to prevent the meeting taking place. Indeed, the ground in front of the building had been cleared of snow on the eve of the meeting, which was entirely orderly. Afterwards, the president of the academy, Guri Marchuk, received a delegation of those present at the meeting.

The issue that has exercised the scientific public is the nomination on 18 January of candidates from the academy to the Congress of People's Deputies. As a result of the voting at that extended meeting of the academy's membership, the candidates who had received most support at meetings of institute personnel were found to be missing from the final lists. Rank-and-file scientists say the outcome shows their wishes have been neglected. The five resolutions passed at the meeting on 2 February show a deep crisis of confidence in the academic community.

The most remarkable occurrence then was the contribution of Academician Vladimir Kudryavtsev, elected a vice-president of the academy only last year and put up at the meeting as the academy's spokesman. As chairman of the disputed election meeting, he could hardly have expected a hearty welcome from his critics, and there were several attempts to shout him down. He deserves praise for his determination to speak out.

"Read the slogans", he said at one point, "but I agree with most of them." " 'No to functionaries from science!' I also say 'No!' 'Scientists for Gorbachev and perestroika!' I also say 'Yes!' 'Let Sakharov, Sagdeev and Likhachev be our deputies!' I voted for them myself!"

Kudryavtsev went on to say that he agreed with the demand that the academy should be restructured and that a public association of scientific workers should be established. Saying that relations between the academy and its institutes should be more democratic, he asked for the meeting's help to this end.

On the outcome of the voting on 18 January, Kondryavtsev said he shared the meeting's "discontent and regret". Several days earlier, the praesidium of the academy had said that the results of the voting had come as a bolt from the blue for everybody. Academician Marchuk said that he had also voted for Sakharov and Sagdeev.

As an observer at the plenary meeting, I had the impression that many leaders of the academy forgot to which body they were electing people, and by what principles and criteria. Counting the number of institutes backing one nominee or another should not have been irrelevant. Many failed to listen to Sakharov's appeal at the beginning of the meeting that there should be more confidence in the institutes' proposals. Although the voting was by secret ballot, the leadership must assume responsibility.



Post-glasnost *Sakharov now free to travel — en route from Italy to Canada (AP).*

It is understood that the central electoral commission has said that there was "no violation of the law that would justify cancellation". But this view was not generally accepted by the commission, which agrees that the law should be further refined and made specific.

The affair is not yet over. One of the demands of the public meeting on 2 February was that the 23 candidates nominated on 18 January should now withdraw. There is also a proposal that the elections due in March should be boycotted to allow voting on a list drawn up by the institutes.

Sakharov, the front runner in the electoral process until 18 January, told me that he has now been nominated by several districts in the Moscow region and is not fully aware "who has appointed me, and where". Several other candidates disappointed at the academy elections, Academicians Dmitry Likhachev and Gavriil Popov, for example, have also been nominated by other constituents. But the fate of the academy's representation in the chamber of deputies will not be known until the special conference of the academy from 18 to 22 March. **Yuri Kanin**

Novosti

Sagdeev declines to stand in Kazan

Moscow

THERE has been a further escalation of the conflict within the Academy of Sciences over the nomination of candidates for the election of people's deputies. As *Nature* went to press this week, our Moscow correspondent Yuri Kanin learned that Academician Roald Sagdeev has declined to stand as a candidate for the city of Kazan, saying that he will instead "support with all my vigour" the staff of academy institutes in their "just fight against the scientific establishment".

● Kanin writes: Sagdeev has been backed by staffers at 25 research institutes of the academy, but did not make it to the list of candidates put forward by the general meeting of the academy. Instead, he was nominated by the ethnic-territorial district number eleven of the Russian Federation, which consists chiefly of the city of Kazan.

In a cable to the electoral commission of the district, Sagdeev thanks the electorate for having nominated him, hopes that they will instead nominate local followers of Gorbachev's *perestroika* but declined to stand himself.

"I feel obliged", the cable says, "to support with all my vigour the staff of many institutions of the academy in their just fight against the members of the 'scientific establishment' sitting pretty at the academy's headquarters".

In an earlier interview, Academician Vladimir Kudryavtsev, the chairman of the general meeting of the academy at which the disputed nominations were made, explained that he had attended the public meeting in the academy's grounds on 2 February in a personal capacity. Saying that he had been surprised and disappointed by the outcome of the election, he denied the suggestion at the 2 February protest meeting that the list of those nominated had been drawn up by the praesidium of the academy.

Kudryavtsev also explained that while the praesidium has expressed regret at the outcome of the election, it could not have invalidated the result without flouting the procedure adopted at the outset of the election meeting. "Unless we learn to honour the laws, we will return to the times when we could only talk about democracy, while there was none".

Yuri Kanin

Novosti

Shroud irradiated with neutrons?

Sir — If the shroud of Turin is in fact the burial cloth of Christ, contrary to its recent carbon-dated age of about 670 years (*Nature* **335**, 663; 1988 and **337**, 611; 1989), then according to the Bible it was present at a unique physical event: the resurrection of a dead body. Unfortunately, this event is not accessible to direct scientific scrutiny, but the image on the shroud, which still cannot be duplicated, appears to be a scorch, indicating that the body radiated light and/or heat. It may also have radiated neutrons, which would have irradiated the shroud and changed some of the nuclei to different isotopes by neutron capture. In particular, some ^{14}C could have been generated from ^{13}C. If we assume that the shroud is 1,950 years old and that the neutrons were emitted thermally, then an integrated flux of 2×10^{16} neutrons cm^{-2} would have converted enough ^{13}C to ^{14}C to give an apparent carbon-dated age of 670 years.

This flux of neutrons should have other measurable consequences. The neutron irradiation would probably not have been uniform, for example, so the $^{14}C/^{13}C$ ratio should vary in different parts of the shroud. In addition, other unstable isotopes should have been formed. Several of these isotopes have half-lives long enough that they would still be present, yet short enough that they are not found naturally.

The unstable isotopes most likely to be found in the shroud are ^{36}Cl and ^{41}Ca. The presence of either would confirm that the shroud had been irridated with neutrons. An accurate measurement of the ratio of either ^{36}Cl to ^{35}Cl or ^{41}Ca to ^{40}Ca (see table) would test the prediction of an integrated neutron flux of 2×10^{16} neutrons cm^{-2}. This may not be possible, however, because contamination with new sources of chlorine or calcium may have occurred from washings or other sources since the irridation took place.

THOMAS J. PHILLIPS
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HEDGES REPLIES—The processes suggested by Phillips were considered by the participating laboratories. However, for the reasons given below, the likelihood that they influenced the date in the way proposed is in my view so exceedingly remote that it beggars scientific credulity.

(1) No plausible physical mechanism has been proposed to explain how the resurrection was accompanied by a significant neutron flux. If a supernatural explanation is to be proposed, it seems pointless to make any scientific measurement on the shroud at all.

(2) Assuming a 'scientific' (but not yet articulated) explanation for the neutron flux, it is an amazing coincidence that the neutron dose should be so exactly appropriate to give the most likely date on historical grounds. (Arguably a total of 10^{28} neutrons (the number in a human body) would be available. Using Phillips' figures, this would be sufficient to impart a date of 100,000 years into the future. To produce a date within 100 years of the first recorded history of the shroud implies that the dose has been 'fine-tuned' to better than one part in a hundred million.)

(3) In fact, the dose proposed by Phillips is much too high, as he has not included the neutron capture by nitrogen in the cloth. A not untypical N content in linen is 1,000 p.p.m., for which a thermal neutron flux of 2×10^{13} cm^{-2} (that is, 1,000 times less) would be appropriate. This does not change the basic argument, but changes the chemical implications (even without recoil effects). The three dating laboratories used different types of chemical pretreatments, (for example at Oxford we purified the cellulose), yet obtained equivalent results. This shows that any ^{14}C formed by neutron irradiation behaves chemically in the same way as the original ^{14}C. This is inherently unlikely because the original nitrogen is in a chemically quite different environment.

(4) As Phillips comments, one might expect that the 'irradiation' would be non-uniform. The three samples were contiguous, but at least on a local scale of 10 mm, any such variation was less than 1 per cent.

The lower neutron flux would reduce the conversion of ^{35}Cl to ^{36}Cl, but should still give a ratio significantly above background (if there has been no loss or gain of Cl since). Electron spin resonance signals from the 'irradiation' might also persist. However, such measurements are unlikely to confirm the presence or absence of neutron irradiation with absolute certainty. This, I fear, will not be achieved in a finite number of tests. If we accept a scientific result, we must exercise a critical notion of the probabilities involved. If we demand absolute certainty, we shall have to rely on faith.

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Properties of selected parent/daughter isotopes

Parent isotope	Natural abundance (per cent)	Neutron capture cross-section (barns)	Daughter isotope	Half-life (years)	Predicted parent/daughter ratio
^{13}C	1.11	0.0009	^{14}C	5,730	1.2×10^{-10}
^{35}Cl	75.77	43	^{36}Cl	3.0×10^{5}	8×10^{-7}
^{40}Ca	96.94	0.40	^{41}Ca	1.0×10^{5}	8×10^{-9}

Predicted parent/daughter ratios (right-hand column) are for a new cloth irradiated with 2×10^{16} neutrons cm^{-2}. For $^{14}C/^{13}C$, the ratio is about 17 per cent higher than normal.

Why reprints are necessary

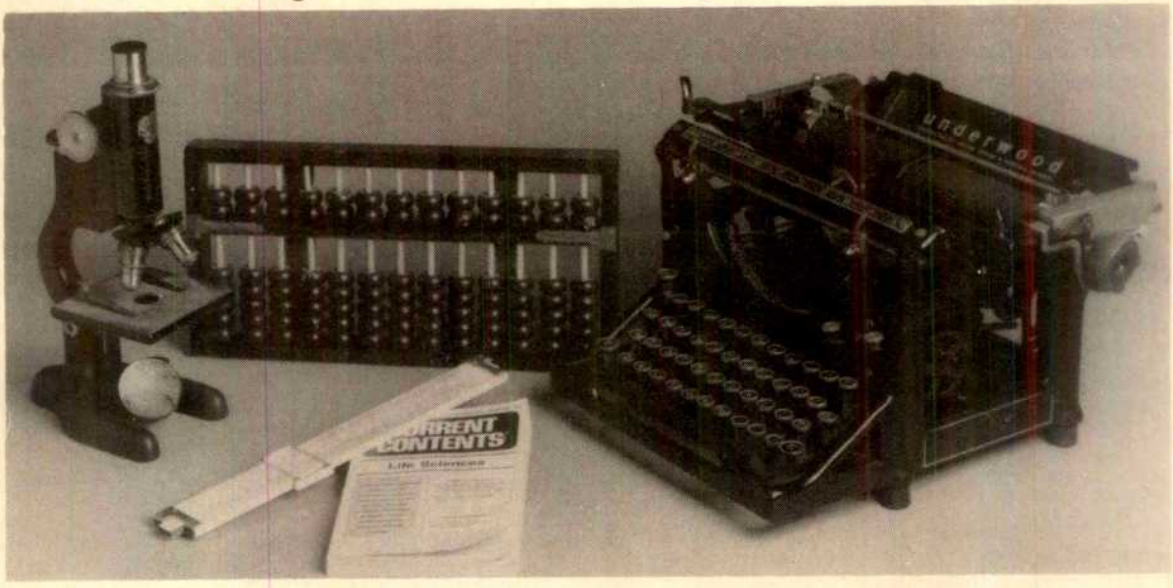
SIR—Ivor Smith (*Nature* **336**, 708; 1988) complains of receiving reprint requests for a short paper and claims that "the cost of sending for this reprint and the time involved are appreciably greater than walking to the library and making a single photocopy for one's own use". He further suggests that photocopying articles, rather than sending reprint requests, is the "sensible approach".

Perhaps he is correct, if one reads only one short article each week. But in many scientific fields, including my own, one comes across 20–30 articles a week. The cost and time spent walking to the library, finding the articles and photocopying 30 articles is significantly greater than the cost and time required to fill out reprint requests. The volume of research articles is such that only with the time-efficient method of perusing *Current Contents* and requesting reprints of significant articles do I have a hope of having the time to actually read the articles. Thus, for many scientists, the reprints are requested on the basis of the title of the article, which is why it seems to Smith that the requesters have not yet read the article (and apparently will not read the article, considering that they do not receive the reprint).

If it is true that the "European reprint is a disappearing commodity", then perhaps the numbers of scientists reading European articles will be reduced, and European scientists themselves will be even more hard-pressed to keep up with the literature. This may be another funding-related factor which contributes to the decrease in scientific productivity in Britain, lamented in *Nature* over the past two years. Reprints may be expensive, but I believe that they remain the most time-efficient method of article acquisition, and will remain so until the development of 'electronic' journals.

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Supernova springs new surprise

What seems to be the pulsating star left by the supernova whose explosion was observed two years ago has surprised everybody and could, if confirmed, stand theory on its head.

ASTRONOMERS have been grow ing a little anxious in the past few months, wondering when the pulsar left by the explosion of supernova 1987A would emerge from the cooling remnant. The longer they waited, the more they suspected that the pulsar would be feeble and undistinguished. Instead, if observations announced last week stand up to scrutiny, astronomers are faced with three surprises — and a raft of theoretical puzzles.

The bare facts, given in IAU Circular No. 4735 by John Middleditch of Los Alamos National Laboratory, Carl Pennypacker of Lawrence Berkeley Laboratory and many others, are that optical variations between magnitudes 18 and 19 were detected during a 7-hour observing period on 18 January with the 4-m telescope at Cerro Tololo Observatory in Chile. From these observations comes a pulsar with a period of half a millisecond, making it the fastest known. And there is also an 8-hour variation suggesting that the pulsar is a member of a binary system.

The three surprises are these. How can a new-born pulsar have a sub-millisecond period, when it has been thought for years that pulsars are born rotating perhaps ten times a second? Why is the pulsar's magnetic field so small, which it must be because luminosity goes as the square of the field strength and the fourth power of the rotation rate, and because the pulsar is certainly no brighter than the much more slowly rotating Crab pulsar? And how has it acquired a companion which, had it been present at the time of explosion, would have been sitting in the outer layers of the giant progenitor star?

Most pulsars have periods of some modest fraction of a second; the few with millisecond periods belong (with a couple of exceptions) to binary systems. So, the story goes, all pulsars are born rotating slowly, and spin down as they age. But a pulsar in a close binary can accrete mass from its companion, and be spun up to a millisecond period as mass spirals in, conserving angular momentum. The handful of isolated millisecond pulsars are supposed somehow to have lost their companions, perhaps through encounters with other stars or, as in the celebrated case of PSR1957+20, by blasting them away by radiation.

This tidy picture absolves astrophysicists who calculate the dynamics of supernovae explosions from the immense trouble of dealing with rotation, but the 1968.629-Hz periodicity of the new pulsar changes that picture. Its rotational energy is about the same as its gravitational binding energy, which means that it is on the point of being physically flung apart. (For some theoretical nuclear equations of state, it ought to fly apart, which is another problem.) Moreover, the energy of the explosion was only a small fraction of that now tied up in rotation, which means that refinements of the distribution of angular momentum in the progenitor yield order-of-magnitude effects in the explosion dynamics.

For Stan Woosley of Lick Observatory, who has exploded many stars by computer, the problem is that no one knows the distribution of angular momentum in a giant star, or how to calculate angular momentum transport through a convective turbulent medium evolving on a millisecond timescale.

The angular momentum of a giant star, conserved through collapse to a neutron star, is enough to create a millisecond pulsar. But hoping to follow the collapse in detail, and to make useful predictions about the outcome, is close to impossible.

Woosley is understandably waiting to see whether the observations are confirmed before he attempts to explain the new pulsar, and is not averse to models in which the pulsar was born slow two years ago, but has been spun up by accretion since then. But others, such as Andrew Fabian, of the Cambridge Institute for Astronomy, are sceptical. Although dumping about half a solar mass of material onto a fledgling neutron star could spin it up to a millisecond period, the energy generated would be far above the Eddington limit (meaning that radiation pressure would be enough to repel the infalling material).

The Eddington limit can be evaded, for example by supposing gas to accrete at the equator and radiation to emerge from the poles, but this requires special pleading. And the extra gas must come from somewhere: if the supernova explosion has to be such that the core collapses, the atmosphere is blown off yet a little gas is left stranded for subsequent accretion, the pleading becomes even more special.

Like its rapid rotation, the low magnetic field of the new pulsar will probably just have to be accepted for what it is. The elementary assumption that a pulsar obtains its luminosity from electrons accelerated by the rotating magnetic field leads to a simple relation between brightness, magnetic field and rotation rate. The dipole field strength of the new pulsar can be no more than about 10^9 gauss, whereas 10^{12} gauss is what astrophysicists would have guessed.

If the rapid rotation and low field are odd, the apparent 8-hour orbital period is bizarre to the point of incredibility. The companion apparently has the mass of Jupiter and an orbital radius putting it inside what would have been the outer hydrogen layer of the progenitor.

While it is on the fringes of possibility that a condensed object might have lived for a little while in the cool atmosphere of a giant star, it is quite impossible that any such object could have survived the explosion. Some unusual hypotheses come to mind: if the pulsar was spun up by recent accretion, then the companion could be a blob accreting material; if the core was rotating very fast as it collapsed, then it might have passed through a "dumb-bell" phase and fractured into two pieces.

The recent case of PSR1957+20, in which radiation from a pulsar is presumed to be eroding a nearby companion, suggests to Edmund van den Heuvel of Amsterdam University a similar but more extreme history for the pulsar in SN1987A. Because of its very rapid rotation, the newly formed pulsar may have the power to boil away a companion of one solar mass in a matter only of years, rather than the millions of years taken by PSR1957+20. To do this, a much stronger magnetic field than the simple-minded limit allows is needed but because the system is evidently evolving on a timescale of years, even months, the usual arguments may be quite wrong.

The reality of the 8-hour periodicity is certain to be questioned. But Pennypacker and his colleagues are the best in this business, and they spent several weeks analysing their data because they too were taken aback by what they found. New observations are scheduled two weeks from now. The new pulsar may be a freak but van den Heuvel at least seems prepared for a wholesale revision of ideas: the notion that pulsars are born slow may turn out to be an unfounded guess, an unwarranted extrapolation to the moment of birth from 1000-year old objects such as the Crab pulsar. The infancy of the new pulsar will be followed minutely, and will put adulthood and old age in a new light.

David Lindley

Retroviral proteinases

A second front against AIDS

Tom Blundell and Laurence Pearl

Of all the main infectious diseases in man, those caused by viruses are the greatest challenge to the development of effective drugs. Retroviruses such as human immunodeficiency virus (HIV) code for three enzymes of their own: reverse transcriptase, integrase and proteinase. Attempts to develop drugs have so far focused on the inhibition of the viral reverse transcriptase. This enzyme is the target of AZT, the most effective anti-HIV drug in clinical use. Although screening compounds for antiviral activity could turn up useful drugs, it is knowledge of the three-dimensional structure of the virally encoded enzymes that should allow *de novo* design of compounds with high specificity and low toxicity. The basic data for receptor-based design of a new generation of anti-AIDS drugs are provided by two new X-ray analyses of retroviral proteinases (or proteases). Last week, Wlodawer and colleagues reported the structure of Rous sarcoma virus (RSV) proteinase, and on page 615 of this issue[2], Navia and co-workers report the structure of the enzyme of HIV-1 itself.

Tang *et al.*[3] in 1973 observed an unusual sequence of three amino acids, Asp-Thr-Gly, that occurs at both of the two aspartate residues at the active site of pepsin. Subsequently it was discovered[4] that aspartic proteinases such as pepsin have a bilobal structure with a well-defined cleft. A pseudo dyad relates two similar domains with Asp-Thr-Gly sequences occupying topologically equivalent positions and hydrogen-bonded at the centre of an extended active-site cleft. Both studies[3,4] suggested these proteins evolved from an ancestral dimeric enzyme. Toh *et al.*[5] found clues for a present-day relative of this ancestral aspartic proteinase by identifying a highly conserved Asp-Thr-Gly sequence in retroviral sequences in regions thought to encode the proteinase responsible for maturation of the virus. Alignment of consensus sequences for the retroviral proteinases with the sequences of the two lobes of pepsin-like enzymes[6,7], together with the finding that there is weak inhibition of retroviral proteinases by pepstatin[8], indicated that the retroviral enzyme would be dimeric and have a pepsin-like fold. These observations suggested that retroviral inhibitors, perhaps useful for the treatment of AIDS, might be achieved using the technology previously developed for the design of anti-hypertensive inhibitors of renin[9], an aspartic proteinase related to pepsin.

The newly described crystal structures of the two retroviral proteinases — RSV at high resolution[1] and HIV-1 at medium resolution[2] — confirm the prediction of a dimeric structure with a fold similar to other aspartic proteinases[6]. Each enzyme comprises two subunits, each containing a mixed β-pleated sheet of two intertwined motifs of four strands (a, b, c, d and a′, b′, c′, d′) related by a pseudo dyad as shown in Fig. 1. The active-site Asp-Thr-Gly or Asp-Ser-Gly sequence occurs on the wide loop (c, d) which is crossed by strand d′ to give a PSI-like structure. Although only the RSV, and not the HIV-1 structure is reported at resolution high enough reliably to indicate the side-chain conformations, it appears that both

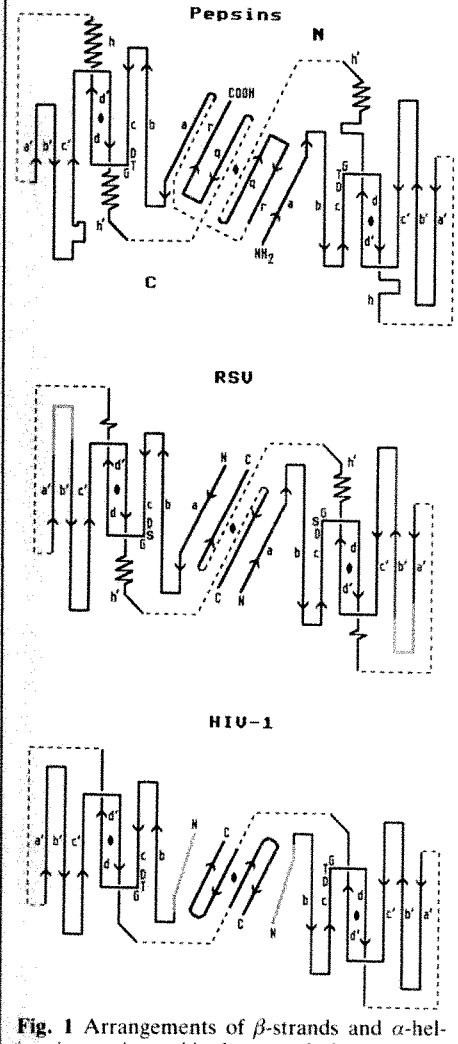


Fig. 1 Arrangements of β-strands and α-helices in pepsins and in the retroviral proteinases of RSV and HIV. In the three-dimensional structures the sequences DT(S)G on strands c are hydrogen-bonded together at the centre of the active-site cleft around the dyad (see Fig. 2). Regions that are unclear in the electron-density maps are shown in grey; dashed lines indicate connections that are short in three dimensions.

enzymes have the characteristic aspartates hydrogen-bonded together at the centre of the active-site cleft[1,2]. The serines or threonines stabilize the symmetrical dimer in an exactly analogous way to pepsin by forming a buried 'fireman's grip' between the subunits[10].

The variations in three-dimensional arrangements of the carboxy-terminal strands and the adjacent amino-terminal strand shown in Fig. 1 reflect the shorter sequences of the retroviral proteinases when compared with the pepsins. Nevertheless, it is suprising that there are differences between the two viral enzymes, including the absence in the HIV enzyme both of a helix in a relatively well-conserved region, h′, and of a clearly defined amino strand. Although these strands are involved in subunit interactions within the dimer, the relative positions of the subunits are well conserved between retroviral proteinases and pepsins. Furthermore, the variations at the chain termini occur mainly on the opposite side to the cleft, and therefore will not greatly affect the pockets that define enzyme catalytic activity or substrate specificity.

The extended hairpin a′, b′, which forms the 'flap', also varies greatly, and there is no equivalent of the invariant Tyr 75 of pepsins that may stabilize the transition state[9]. In fact, the end of the flap of RSV proteinase cannot be seen in the electron-density map even at 2 Å resolution[1], indicating high mobility. By contrast, the two flaps of the HIV proteinase are hydrogen-bonded to neighbouring molecules[2], as in penicillopepsin[11], and so, although they are clearly visible, their position may not be relevant to the enzyme in solution. In any event, it seems that the function of one or both flaps in the retroviral enzymes is to interact with the substrate and to exclude water from the substrate-binding site.

Comparisons and analogies between the structures of retroviral and aspartic proteinases should be useful for the design of HIV proteinase inhibitors. Although the two subunits are completely symmetric in retroviral proteinases, the substrate is asymmetric. Consequently, identical regions in the two subunits must interact with different regions of the substrate on either side of the scissile bond; this may induce asymmetry in the retroviral proteinase itself. By analogy with the pepsins, the substrate binding appears to be achieved by exploiting a weak pseudo symmetry in the substrate[9]. Thus, side-chains of P_1 and P_1' probably bind in identical hydrophobic pockets (Fig. 2). The chain is likely to be positioned by hydrogen bonds between the main-chain amino functions of P_1 and P_2' and the carbonyls of Gly 27 of each subunit of HIV proteinase. A further main-chain interaction at P_3 with the main-chain amide and side-chain oxygen of Thr 219 in

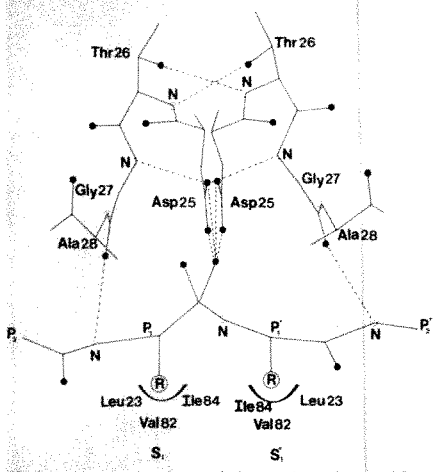


Fig. 2 Arrangements of the active-site residues Asp-Thr-Gly-Ala at the active site of retroviral proteinases, together with a model of the transition state of the substrate based on X-ray studies of analogous pepsins. Dashed lines indicate possible hydrogen bonds. The scissile bond lies between P_1 and P_1'.

pepsins[7,12,13] may be replaced by main-chain amide and side-chain oxygen of Asp 29 in HIV proteinase. The almost invariant Arg 87, which has no analogue in pepsins, appears to be ion-paired to this highly conserved aspartate. Most excitingly, the perfect symmetry of the retroviral proteinases implies that their inhibitors could also have perfect 2-fold symmetry, giving a considerable gain in affinity for the inhibitor relative to the substrate transition state.

What do the crystal structures tell us about the biological function of the proteinase in viral assembly and maturation? The molecules observed in the crystals are not the initially translated forms of the enzymes, but derive from larger precursors. In RSV, the proteinase is synthesized as part of the Gag polyprotein, whereas in HIV it is encoded as part of the Gag–Pol fusion polyprotein. The Gag and Gag–Pol precursors are the native substrates for the viral proteinases. As there is no evidence for involvement of cellular enzymes in the proteolytic processing of Gag and Gag–Pol precursors — in contrast to the processing of the Env precursors — the proteinase must be responsible for the cleavage of its own precursor.

Because the dimer is the active form of the enzyme, the question arises as to whether the precursor cleaves itself or whether it is cleaved by another dimer. Navia *et al.*[2] in this issue suggest that in the HIV proteinase the disordered amino-terminal strands are sufficiently flexible to fold into the active-site cleft so that they can be cleaved by an intramolecular reaction, similar to the mechanism that occurs in pepsinogen[14]. In the RSV structure, however, this strand appears to be well ordered. It is unlikely that the carboxy-terminal strand of either viral enzyme could be cleared intramolecularly without disrupting the integrity of the dimer.

It is the precursor that forms the initial budding 'immature' viral particles, and careful spatial and temporal control of the proteolysis must be important for the production of mature infectious virions. Navia *et al.*[2] suggest that proteinase dimers will be active in a precursor form before budding, but will not cause premature cleavage of membrane-associated Gag precursors because of effective dilution in the large volume of the cell.

There are other mechanisms for control of the initiation of the proteinase cleavage. Aspartic proteinases as a class are optimally active at acidic pH and retroviral proteinases behave similarly. Mammalian aspartic proteinases such as pepsin are synthesized as inactive zymogens and are activated by a drop in pH[15]. A similar mechanism may operate in retroviruses by progressive acidification after budding or by budding into exocytotic vesicles.

Evidence from other retroviruses[16] suggests that proteinase activity might also be dependent upon the correct stoichiometry of the Gag and Gag–Pol precursors and their correct assembly in the icosahedral capsid of the budding virion. It is possible that the precursors occupy special positions in the icosahedral symmetry of the immature virus particle and that the molecular 2-fold axes that generate the active proteinase dimers coincide with the 2-fold axes of the icosahedral immature capsid. □

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Microelectronics

Feedback cooling of currents

Sean Washburn

JUST as feedback can be used to correct errors in the output voltages of amplifiers, it can also be used to remove noise from the current through a resistor — including the 'Johnson/Nyquist' noise that results from the thermal motion of electrons[1]. Such a fed-back amplifier behaves as a refrigerator cooling the electrons in a resistor connected to it. This principle has been recognized since the 1940s (ref. 1), but largely ignored because the cooling power available from such refrigerators is minuscule. John Price points out in a new paper[2] that the method might be practical for cooling the currents in (but not necessarily the materials of) the microscopic circuits that are typical of modern electrical engineering and recent studies in transport physics. Because of an analogy to the method used in collimating particle beams in high energy physics experiments, he names the process 'stochastic cooling'.

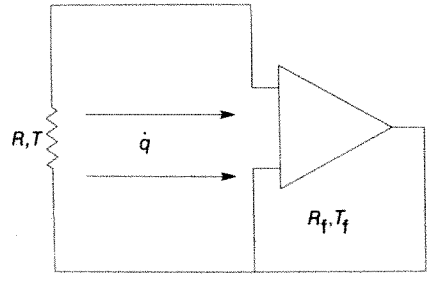


Fig. 1 Circuit diagram of the essential elements of the feedback refrigerator.

The thermal energy associated with a non-zero temperature in materials is distributed not only among the available vibrational motions of the lattice, but also in random agitations of the electrons. These give a finite limit to the precision with which currents can be detected, and appear as noise limiting the sensitivity of any electronic circuit. A brute-force method to reduce the noise is to cool the whole electronic device, say, in liquid helium. But the method discussed by Price represents a more subtle approach to cooling the electrons alone.

In very small, thermally isolated resistors (typical metal wires with volumes less than about 1 μm^3), Price's feedback refrigerator might be sufficiently powerful to lower the electron temperature in the wire from $\lesssim 10^{-2}$ K (a temperature within reach of conventional refrigerators) to less than 10^{-6} K. If accomplished, this would mark a lowering of electron temperatures by more than an order of magnitude below the current record, which is about 10 μK obtained by nuclear demagnetization (cooling of the nuclear-spin temperature)[3,4].

The cooling circuit (Fig. 1) uses an 'active resistor' R_f to absorb energy from a passive resistor R. The noise current from

the resistance R at temperature T is then cancelled by the feedback loop just as other error signals would be cancelled in a more conventional application. A current of heat flows from R to R_t and is dissipated there. Under operating conditions ($T >> T_t$), the heat current or cooling power is $\dot{q} = kT\Delta f$, where kT is the thermal energy of the electrons in R and Δf is the bandwidth of frequencies over which R and R_t communicate. Practically speaking, Δf cannot be much greater than 10 gigahertz (GHz), which means that $\dot{q}$ will not exceed 10^{-12} watts (W), and it is likely to be much smaller. The typical cooling powers from dilution refrigeration or demagnetization are orders of magnitude greater (in the microwatt range) but at concomitantly higher base temperatures[3].

To obtain the lowest electron temperatures, one must employ an amplifier with very low intrinsic noise in making R_t. The best candidate is the superconducting quantum interference device (SQUID) comprising a loop of superconducting material broken at two points by tunnel barriers, especially the double-interferometer or direct-current SQUID. The amplifier works by phase-locking the wavefunctions of 'Cooper' pairs of electrons (which are the basis of superconductivity) across the tunnel barriers. In this case the feedback circuit (operated typically at 4 K) is likely to limit the base effective noise temperature of R_t, but this is quite small in some amplifiers — as low as $T_t \leq 1$ μK (ref. 5) — and the fundamental limit is lower. The output noise from the feedback amplifier could be coupled (very weakly) to another SQUID and be read as an indicator of the electron temperature in the wire. The usefulness of the technique depends on surmounting the two design problems (which Price acknowledges) of reducing the heat flow into the sample below the cooling power of the refrigerator and of making a suitable coupling circuit between the passive resistor and R_t.

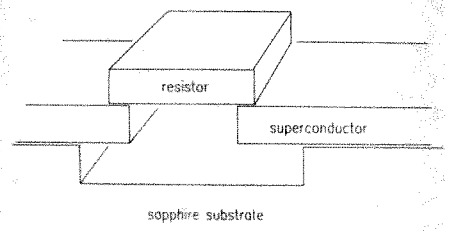


Fig. 2 A possible realization of the refrigerator that thermally isolates the resistor.

To imbed a thermally isolated resistor into the circuit, Price suggests the use of superconducting contacts. This might be accomplished as in Fig. 2 where the metal wire forms a bridge between the superconductors, which in turn are connected to the rest of the feedback refrigerator. The sapphire substrate is a strong enough thermal conductor to allow a conventional refrigerator to lower the temperature of the whole circuit into the millikelvin range. The feedback refrigerator would then have to surmount the heat leaks shown in Fig. 3. The greatest heat leak into the wire is through the thermal conductance of the superconductors, and at temperatures far below the 'energy gap' of the superconductor this leak is typically[3] (although not always[6]) quite small. At such temperatures, essentially all of the electrons in the superconductor are bound up in Cooper pairs that carry no heat at all.

The only remaining thermal conductance is through vibrations of the lattice (phonons) of the superconductor to the lattice of the wire, and then into the bath of normal electrons in the wire. Again the low temperatures reduce this drastically; the thermal conductivity of the phonons in the lattice falls as the cube of the temperature. The coupling of the energy from the phonons to the electrons in the metal also falls as a power law in the temperature. So overall, at very low temperatures, the electrons are likely to be largely decoupled from the lattice temperature (see Fig. 3).

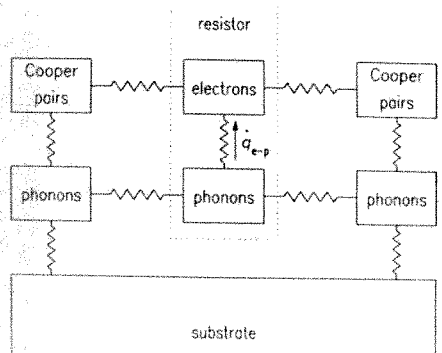


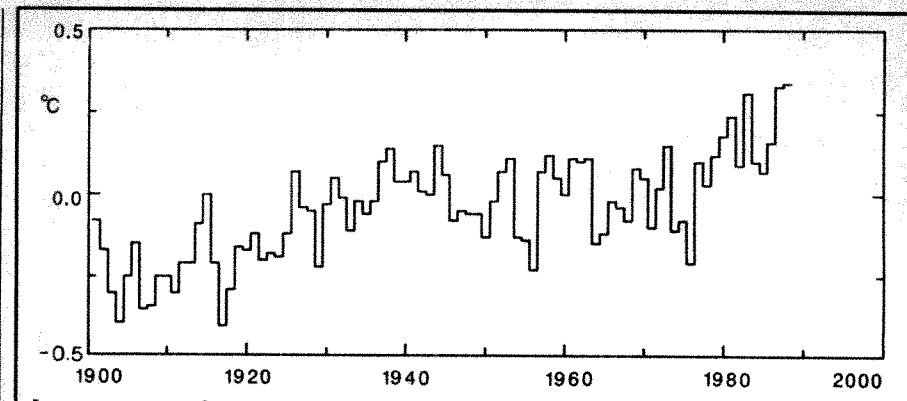
Fig. 3 The heat conduction paths into the resistor in Fig. 2. The circuit elements indicate thermal baths and thermal conductances among them. Measurements[7] indicate that the coupling between the electrons and the lattices is poor even at temperatures ≥ 10 mK. Typically the heat leak from the lattice to the electrons is $\dot{q} = \alpha VT^z$ (V, volume). Because the coupling strength α is rather small[7,8] and the exponent z is large (3–5; ref. 8), this heat leak should be small, so that cooling of the electrons separately from the lattice should be possible.

The problem of the coupling between the passive and active resistors is thorny. It must limit the bandwidth of frequencies between which R and R_t communicate to the range in which R_t exhibits low noise and is capable of cooling, but it must dissipate no energy. Any dissipation in the coupling is noise (increased temperature) that can be dissipated in R. The coupling must therefore comprise purely reactive elements linked by superconducting wires. This in turn causes further complications. The use of superconducting contacts requires that a magnetic field be applied to quench any proximity-effect superconductivity in the resistor. And the magnetic flux in the superconducting elements can lead to dissipation providing a further heat leak.

Assuming that the difficulties in the connecting circuitry can be overcome, Price shows that for reasonable values of resistor size and phonon temperature a conservatively specified direct-current SQUID (noise levels far in excess of the best yet obtained[5]) should be able to cool the electrons in, say, a thin copper film to less than 1 mK. For extreme design criteria, the base temperature might be orders of magnitude lower. □

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LAST year was the warmest year since records began. Global temperature records analysed at the UK Meteorological Office and the University of East Anglia show that the global average temperature for 1988 was 0.34 °C above the long-term (1950–1979) average and that eight of the nine warmest years this century have occured in the 1980s. Whether this apparent warming trend is a consequence of the 'greenhouse effect' is moot; as variations ranging from the daily changes in weather to the long-term glaciations show, global temperatures fluctuate considerably owing to natural causes. But the observed warming is consistent with model predictions of the greenhouse effect.

Philippa Lloyd

Multiple sclerosis

Relationship to a retrovirus?

Byron H. Waksman

E. PREMKUMAR Reddy and colleagues at the Wistar Institute in Philadelphia and the University of Lund in Sweden, in the 27 January issue of *Science*[1], describe the identification of DNA sequences corresponding to human T-lymphotropic virus (HTLV-I) in the peripheral blood mononuclear cells of six Swedish multiple sclerosis patients. They also describe their failure to find similar sequences in all but one of twenty healthy controls (ten Swedish and ten North American). Some but not all of the patients are reported to have antibodies against the virus in their blood.

This report is an extension of studies published by the same group 3 years ago[2] in collaboration with Robert Gallo's group at the National Cancer Institute. The new work[1] involves the use of the powerful polymerase chain reaction (PCR), which detects and amplifies low concentrations of viral material, allowing molecular cloning and sequencing of the amplified DNA. The report has, not surprisingly, caused a flurry of excitement in the media, because it suggests a relationship between a retrovirus related to HTLV-I and multiple sclerosis. It does not imply that the retrovirus is the cause of the disease, and the authors do not claim this. But it is possible that these findings will stimulate attempts to develop preventive viral vaccines or therapeutic investigations with drugs which arrest retroviral replication, such as those being developed for the distantly related AIDS virus.

Reddy and collaborators used typical multiple sclerosis patients in their new study[1], many of whom had exacerbating–remitting disease. These were clearly distinct from cases of the slowly progressive, multiple sclerosis-like disease tropical spastic paraparesis, or HTLV-I-associated myelopathy, which is widely distributed in Africa, the Caribbean area and southern Japan. This disease is clearly associated with, and probably caused by, HTLV-I, and clear-cut viral isolation has now been reported by several groups[3,4].

DNA sequences corresponding to both the *gag* and *env* regions of the HTLV-I genome were identified in peripheral blood mononuclear cells from all six multiple sclerosis patients studied by Reddy and collaborators[1]. The results seem persuasive because of the sharp distinction between patients and controls; the concordance of the findings with two different retroviral genes; and the sequencing data provided. At least one other group of investigators, in a study which will shortly appear[5], has succeeded in demonstrating HTLV-I-like DNA sequences in the *pol* and *env* regions in some multiple sclerosis patients but not in normal controls. But when this group used a *gag* primer similar to that used by Reddy *et al.*, all of a series of 21 multiple sclerosis patients, 35 normal controls and 10 cord blood samples gave positive results.

The most interesting possibility raised by the results from both groups[1,5] is that an exogenous retrovirus related to HTLV-I may have a direct causative role in multiple sclerosis. The failure of most laboratories to find antibodies reactive with this virus, and the discrepant antibody finding of Reddy *et al.*[1], speak against this. Another possibility is that Reddy *et al.* are looking at 'endogenous' retroviral DNA sequences, which are widespread in human and other mammalian germline DNA[6]. For some viruses, the complete structure, including *gag*, *pol* and *env* sequences, is often maintained; intact infectious virus is usually not found. At least five known families of retroviruses have been demonstrated in the normal human genome. When supersensitive techniques like the PCR are used, retroviruses resembling HTLV-I can be found in many individuals[6]. But if endogenous retroviral sequences account for the multiple sclerosis results of Reddy *et al.*[1], why were 19 of the 20 controls negative? Results obtained with the PCR technique vary greatly from day to day, and Reddy *et al.*[1] do not report how often they carried out the tests in the multiple sclerosis samples and the controls or even if they ran the multiple sclerosis and control samples concurrently. There is therefore some uncertainty whether the possibility of endogenous sequences is excluded.

Another spectre haunts those who use the PCR technique — the possibility that the test system is contaminated with retroviruses already present in the laboratory. Some take extreme precautions to deal with this possibility, carrying out the PCR in a different laboratory or building than that in which cell separations are done. Contamination can be excluded when the sequences found differ at many positions from all possible contaminants. However, the sequences described by Reddy and colleagues in the multiple sclerosis samples differ only slightly from the comparable sequences of the HTLV-I (derived from patients with tropical spastic paraparesis) studied earlier in the same laboratory. Because the PCR is not an error-free technique, this may be a source of concern.

It is regrettable that the investigators failed to test their techniques on blood cells from patients with diseases related to multiple sclerosis, such as rheumatoid arthritis or juvenile diabetes, or even on mitogen-stimulated normal cells, as the circulating monocytes and lymphocytes of both main subsets are markedly activated in multiple sclerosis, as well as in these other conditions[7]. Cell activation by, for example, phorbol esters results in enhanced retroviral gene expression[6]. Although any possible effect of such activation on the PCR staining of DNA is conjectural, it would be reassuring to know that tests for HTLV-I on activated blood mononuclear cells of such controls were consistently negative.

The pathogenesis of the characteristic inflammatory destructive lesions of multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes, chronic thyroiditis and other similar diseases is explained by many as an immunological reaction to tissue antigen triggered by viral infection (measles, rubella, Epstein–Barr virus) in early life in genetically predisposed individuals[7,9]. This view is based on epidemiological evidence and the study of autoimmune animal models (see my earlier News and Views article[9]), and receives strong support from recent studies of genes associated with susceptibility to multiple sclerosis and of those governing recognition of myelin antigens by T cells. Perhaps an autoimmune reaction can trigger the local expression of exogenous or endogenous retroviral genes, which make a secondary contribution to lesion pathogenesis, but this remains little more than a speculation.

To conclude on a sobering note, one should recall that more than 20 viruses have been incriminated as the possible cause of multiple sclerosis since 1946[10]. In twelve instances, the presumed causative virus was actually isolated from multiple sclerosis patients. Yet none of these candidates has stood the test of time. This underlines the need for further studies in other laboratories to confirm the findings of Reddy *et al.*[1] and for control studies in other neurological and autoimmune diseases. Putting these cautions to one side for a moment, the new findings are among the most interesting to be published in recent years in this difficult field. □

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Structure of galaxies

Origin of the Hubble sequence

M.G. Edmunds

MORE than 60 years have elapsed since Edwin Hubble devised his elegant and concise classification system for galaxies (see figure), but we still do not have a good theory of the mechanisms by which the various types arise. A possible pointer to the correct explanation comes from some recent, albeit rather incomplete, dynamical calculations of Lake and Carlberg[1] performed on a Cray X-MP supercomputer. In their simulations, which incorporate dark matter (a common ingredient in cosmological theories[2]), the authors find that the final form of the galaxies depends strongly on the initial spread of velocities in the pre-galactic distribution of dark matter.

Nearly all galaxies have a spheroidal component — a fairly smooth, three-dimensional distribution of old stars. But spiral galaxies (both barred and unbarred) also show a characteristic thin disk of stars, containing gas and dust. The basic questions are what determines whether a galaxy has a disk, and whether the spheroidal components of spirals are really equivalent to elliptical galaxies.

Although there have been alternative proposals, it is usually assumed that elliptical and spiral galaxies formed at about the same time, and that there is no evolution from one type to another. It has long been realized that, if this is true, the critical process determining the basic type of a galaxy must be the time taken for gas to turn into stars compared with the time required for the initial gas cloud to separate out from the primordial matter during the early expansion of the Universe and collapse under its own gravity. If the gas forms into stars quickly enough, the kinetic energy of the collapse is preserved in the stellar motions. Because the stars rarely interact with each other, a spheroidal or ellipsoidal ball of stars results. If the star formation is slower, then much of the gas can collapse into a disk, dissipating its kinetic energy by collisional shocking of the gas before it is converted into stars. This leads to a disk galaxy, with continuing slow star formation in the disk and a modest spheroidal component comprising the stars which did form during the collapse.

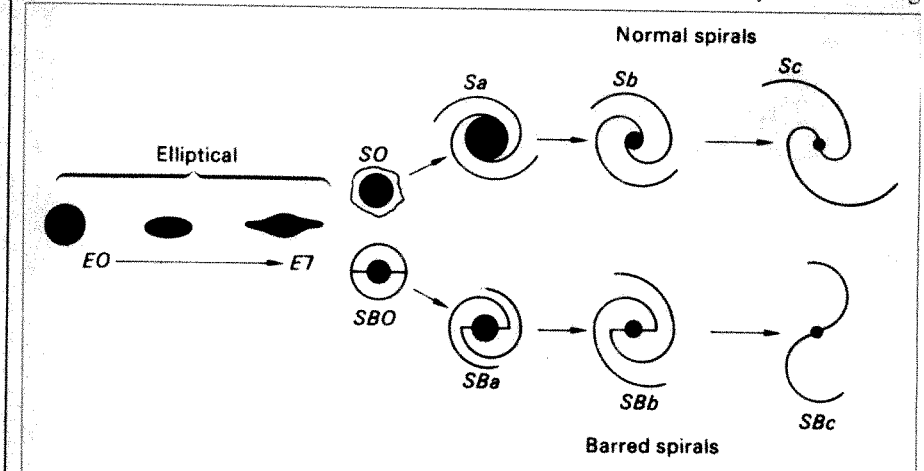
The question then becomes one of deciding what it is that regulates the rate of star formation (local gravitational collapse) relative to the rate of the global dynamical gravitational collapse of the whole galaxy. Thus it has been suggested[3] that star formation is promoted by increased local gas density and inhibited by increased angular momentum per unit mass. The latter suggestion is attractive because spirals in general seem to have higher angular momentum than ellipticals.

Lake and Carlberg[1] have found a regulatory mechanism while investigating a dynamical 10,000-particle model. The model follows the fate of gas which collapses with an underlying distribution of dark matter. The latter, commonly assumed to exist in theories of the evolution of the Universe and inferred to exist from observations of galactic dynamics, interacts in the new simulations through its gravitational potential only. The models have essentially 90 per cent of their mass in this dark, unspecified component — the usual inferred ratio. Star formation is simulated by a simple prescription of what happens when the gas 'particles' (or clouds) collide.

The final form of the distribution of stars and gas after collapse is found to depend critically on the magnitude of the random velocity dispersion initially present in the dark matter. The mechanism seems to work as follows. If the velocity dispersion is low (the matter is cold), the dark matter readily clumps together while the initial cosmological expansion changes to gravitational collapse. This clumping causes extensive violent relaxation — energy and angular momentum are redistributed among the particles (the gas clouds and dark matter) during the collapse. It is the transfer of angular momentum outwards in the cloud which is essential. The result is rapid star formation in the by-now dense and low-angular-momentum central regions, giving an elliptical galaxy. An embarrassment is that a high-angular-momentum gaseous halo is left around the outside, but the authors claim that this could easily be removed by other processes during the galaxy's normal evolution. When the initial velocity dispersion in the dark matter is high (it is warm), the clumpy substructure does not form to such an extent and although some relaxation occurs to give a spheroidal component, densities do not get so great until much of the gas, keeping its high angular momentum, has managed to form into a disk, leading to a spiral galaxy.

One advantage of this new picture is that the redistribution of angular momentum during the formation of the spheroids helps to account for the wide differences in angular momentum content between spirals and ellipticals. The extent of this variation is hard to account for in conventional models[4] in which tidal torques between forming galaxies generate the spin.

What is it that ultimately determines the critical velocity dispersion in the dark matter? Lake and Carlberg propose that the overall mass is responsible, with the rather more massive objects becoming elliptical galaxies. The link is not entirely obvious, and they admit that although total mass has often been invoked[5] as a significant parameter controlling the Hubble sequence, it cannot be the whole story because the different Hubble types have overlapping mass ranges[6]. In determining the details of form, the ratio of spheroid mass to disk mass is obviously important, as the Hubble classification is to some extent based on the degree of spheroid dominance. But published observational determinations of bulge-to-disk ratios are probably much more unreliable than is often realized — photometric separation of disk and spheroid components is rarely unambiguous — and the mass of the spheroid may be as large as that of the disk in many spiral galaxies[7]. Once set by the initial dynamics, the bulge-to-disk



Hubble's classification scheme for the evolution of galaxies. (From ref. 8.)

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ratio may well determine the resulting Hubble spiral class by influencing the formation of spiral structure and star formation rates in the disk. The exact conditions required for the presence of a bar remain rather obscure.

These interesting dynamical models have been investigated over only a very small number of possible parameter variations, and other variables still deserve investigation for their possible effect on the final morphological type. This model implies that the era of galaxy formation occurred at a redshift of about three, beyond which only quasars have yet been observed. This work may show that it is futile to attempt to understand the formation process without taking account of the dynamical effects of the dark matter. □

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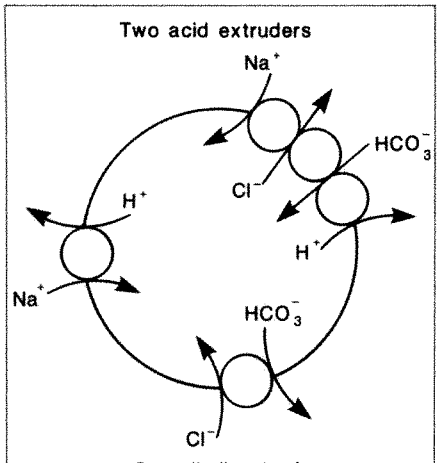
Cell growth factors

Bicarbonate and pH_i response

Roger C. Thomas

MUCH interest was aroused a few years ago by the first report[1] of continuous recording of the intracellular pH (pH_i) response to growth factors in cultured fibroblasts. That the fibroblasts came from human foreskins (*sic*) caused some twinges, but the main interest was the possiblity that growth factors might stimulate growth, at least in part, by increasing pH_i. Moolenaar and his colleagues[1] showed that the pH_i increase was caused by stimulation of Na–H exchange, and later[2] that protein kinase C activation was an intermediate step. But these experiments were done in the absence of bicarbonate, as pointed out at the time in a News and Views article by Boron[3]. On page 648 of this issue[4], Boron and his colleagues now show that the growth factor arginine–vasopressin stimulates HCO_3 transport more than it stimulates Na–H exchange. This result explains the earlier report[5] that in solutions containing HCO_3 and CO_2, there is not only no pH_i increase, but the opposite happens — pH_i decreases.

Boron's group has recently shown[6] that there are three distinct pH_i regulating mechanisms in the renal mesangial cells (related to smooth muscle) which they study (see figure). The first Na–H exchange appears to be the main mechanism for extruding acid from mammalian skeletal muscle[7], and was until recently thought to be the main mechanism for acid extrusion in all mammalian cells. The second, sodium-dependent Cl–HCO_3 exchange, was first identified in squid axons (but without the sodium dependence[8]) and later found in snail neurons[9], other invertebrate preparations[10] and various vertebrate preparations[11–13]. The third mechanism, as first described in cardiac muscle by Vaughan-Jones[14], is one for alkali extrusion — to accelerate recovery from an alkaline load. This mechanism is simple Cl–HCO_3 exchange, as is also found in red blood cells where it is responsible for the Hamburger interchange or chloride shift. (There is in some preparations a fourth mechanism, Na–HCO_3 co-transport, but mercifully it does not concern us here. Because Boron and colleagues in their latest studies did not record membrane potentials[4], they may have missed evidence for this electrogenic carrier.) The three mechanisms shown in the figure have not hitherto been identified all in one cell type, as far as I know. The only mechanism known to work without bicarbonate is Na–H exchange, inhibited specifically by amiloride and related compounds.



Ionic mechanisms involved in pH_i regulation in renal mesangial cells in the presence of bicarbonate.

Given that growth factors stimulate Na–H exchange it might seem unlikely that they would also stimulate the two bicarbonate-requiring mechanisms in renal mesangial cells. But Boron and his co-workers clearly show[4] that all three mechanisms are stimulated. The dominant mechanism after stimulation is the alkali-extruding one. Thus the pH_i increase seen with growth factor in the absence of bicarbonate is under physiological conditions a pH_i decrease. Clearly, the Na–H exchanger is unlikely to be the transmembrane signal transducer originally proposed[1].

Why was the possible importance of bicarbonate ignored for so long? Its crucial role in pH homoeostasis in the intact animal has long been recognized, if poorly understood by most students. Inside cells, it is often the most common anion, and outside it is the second most common after chloride. But in practice, bicarbonate-buffered solutions are difficult to handle. For a stable pH the solution must be equilibrated with a gas mixture containing a known and fixed percentage of CO_2. Such gas mixtures are expensive and tend to vary from cylinder to cylinder. Carbon dioxide readily crosses plastic, especially silicone rubber tubing and leaves solution all too readily when pumped by a peristatic pump, for example. In many optical systems it is simply much easier to avoid any gas at all.

Thus, when it was discovered that cultured mammalian cells could regulate their pH_i perfectly well with an amiloride-sensitive Na–H exchanger, it was widely assumed that bicarbonate would make no real difference. This assumption ignored the significance of bicarbonate in intracellular buffering[10,15], even without taking into account its requirement by pH_i regulators. Because the membrane is very permeable to CO_2, intracellular carbonic acid concentration is effectively clamped. Thus every intracellular bicarbonation can buffer four times better at any physiological pH than any ordinary buffer at its pK. For fast buffering, carbonic anhydrase is needed[16]. The lack of bicarbonate in the original experiment would thus have exaggerated any pH_i change due to stimulation of Na–H exchange.

There are two conclusions from this story. Intracellular pH is probably too fundamental a parameter to act as an intracellular messenger, even for an event as important as the initiation of growth except perhaps in acid-transporting epithelia[17]. As Boron has pointed out[3], it is to be expected that growth factors will stimulate the housekeeping abilities of the cell such as pH_i regulation, in anticipation of the increased metabolic requirement of growth. The second conclusion is that he who works in bicarbonate-free media risks studying cellular and molecular pathology rather than physiology. □

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Palaeontology

New window on Proterozoic life

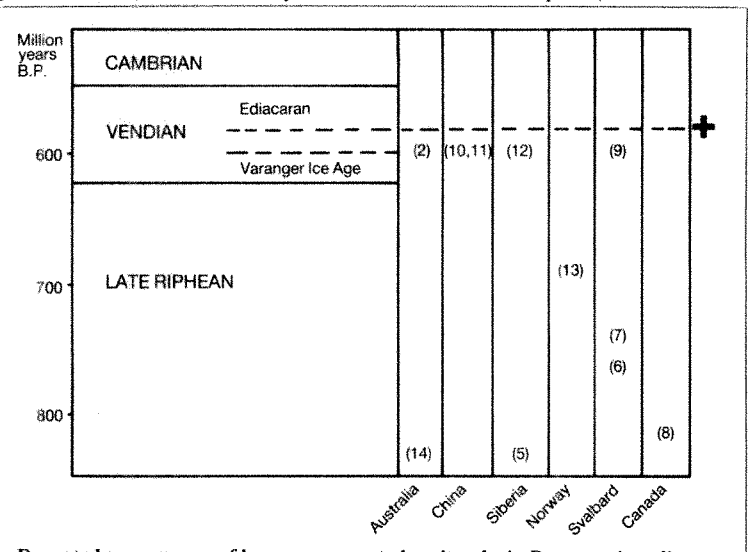
Andrew H. Knoll and Nicholas J. Butterfield

> . . . if my theory be true, it is indisputable that before the lowest Silurian stratum was deposited, long periods elapsed, as long as, or probably far longer than, the whole interval from the Silurian to the present day; and that during these vast, and quite unknown, periods of time, the world swarmed with living creatures.

SURPRISINGLY, it took palaeontologists more than a century to prove the essential correctness of Darwin's speculation[1]. When unambiguously Precambrian microfossils were described from a handful of localities in the 1960s, the minute filaments and unicells preserved in these rocks, notably the Gunflint and Bitter Springs formations, did more than establish the tremendous antiquity of life. They gave rise to a perception of Precambrian communities as vast expanses of cyanobacteria and other prokaryotes, and in younger, Proterozoic rocks, simple, possibly eukaryotic organisms. During the past 20 years, the discrepancy between this well-entrenched textbook notion and palaeontological reality has widened dramatically, as shown by the remarkable new fossil assemblage reported by Zang and Walter on page 642 of this issue[2]. This discovery illustrates the considerable morphological complexity that was attained by late Proterozoic phytoplankton, and underscores the need for careful consideration of stratigraphy, palaeoenvironment and taphonomy (processes of fossilization) in the interpretation of early fossil assemblages.

The new assemblage, found in the 600–650-million-year-old shales of the Pertatataka formation in central Australia, includes about two dozen taxa of spinose, process-bearing or otherwise ornamented acritarchs, many of them extremely large relative to comparably ornamented fossils in younger rocks. (Acritarchs are organic-walled spheroidal microfossils of uncertain taxonomic status. Most are thought to be the cysts of prasinophyte, chromophyte or extinct groups of algae.)

The diversity of this assemblage is unprecedented, but there is a trail of discoveries that leads up to it. Even at the time of the Bitter Springs discovery, populations of morphologically simple acritarchs were known from various Proterozoic shales of the Soviet Union[3]. By the early 1980s, there were enough data on these modestly sculptured acritarchs for a coarse but useful biostratigraphic zonation and to suggest a late Proterozoic diversification of eukaryotic phytoplankton, followed by a latest Proterozoic episode of extinction[4]. Compressions and cellularly preserved remains of seaweeds were also reported, as were vase-shaped protistan microfossils and large, complexly ornamented acritarchs of the type seen in the Pertatataka formation. The latter were first described from approximately 850-million-year-old shales from Siberia[5], and subsequently from slightly younger shales and cherts from Svalbard[6,7] and Arctic Canada[8], as well as from latest Proterozoic rocks of western Svalbard[9], China[10,11] and Siberia[12] (see figure). *Papillomembrana*, a 400–500-μm ornamented vesicle from the approximately 700-million-year-old Biskopås conglomerate, Norway[13], has conventionally been interpreted as a dasyclad alga, but it too probably belongs to this distinctive acritarch grade.



Reported occurrences of large, ornamented acritarchs in Proterozoic sedimentary rocks (references cited in text). The wide geographic and temporal distribution of these fossils suggest the potential for an improved biostratigraphic zonation of late Proterozoic sequences. This distinctive microfossil grade appears not to be represented in Ediacaran or younger assemblages. Absolute dates for microfossil occurrences and stratigraphic boundaries are approximate.

What do these acritarchs reveal about early evolution? The interpretation of fossils begins with systematic palaeontology, and taxonomic choices both reflect and influence the way we think about evolutionary pattern and process. In describing the Pertatataka fossils, Zang and Walter choose generic names that emphasize similarities to younger microfossils. But in our view, a comparison of Pertatataka acritarchs to other Proterozoic fossils is of more interest. Of the 10–15 acritarch genera we believe to be in the Pertatataka assemblage[14], up to a half are unique to this formation, another third are genera previously reported from other Proterozoic formations, and only two or three are most closely related to characteristically Palaeozoic genera — and even here, as Zang and Walter stress, the Pertatataka fossils can be two to three orders of magnitude larger (by volume) than their younger counterparts. Indeed, the most remarkable aspect of these large complex acritarchs is that they do not appear to occur in Lower Palaeozoic rocks. Thus we believe that, together with other recent discoveries, the Pertatataka fossils show that large, complex acritarchs were the characteristic fossils of the later Proterozoic era 900–600 million years ago.

Why is it that assemblages comparable to the Pertatataka were not found, or at least appreciated, earlier? One answer may be that many of these complex microfossils are preserved in subtidal cherts or very fine-grained mudstones, and possibly represent instances of special preservation. Zang and Walter, however, favour an environmental explanation for the unusual composition of their assemblage. They suggest that Pertatataka shales are distal turbidites, a deep-water rock formation little sampled by palaeontologists interested in Proterozoic evolution. The fact that other comparable fossils occur in shallow-shelf deposits, however, suggests that other factors influenced their distribution.

Zang and Walter do not consider biogeographical and stratigraphical distinctions. It is possible, for example, that the morphologically simple acritarchs characteristic of latest Proterozoic shales from

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the Northern Hemisphere (mainly in the Soviet Union and Scandinavia) are not strict time equivalents of the Pertatataka biota. In China, large complex microfossils occur in formations immediately beneath strata bearing Ediacaran metazoans, but Ediacaran assemblages in the strict sense appear to consist only of simple spheroidal acritarchs and very small spiny microfossils (*Micrhystridium* spp.)[11], much as in the Soviet Union.

Whatever the answer, Zang and Walter remind us of the untapped potential of Proterozoic fossils, among other things forcing a reconsideration of the timing of Proterozoic phytoplankton radiations and extinctions. The earlier hypothesis of an end-Proterozoic extinction poorly constrained in time but probably coeval with Varangian glaciation must now be replaced by one of a major turnover just before the Ediacaran radiation. Perhaps the most important effect of the Pertatataka and other recently described fossil assemblages will be to displace the textbook caricature of Precambrian life with a view that emphasizes the biological diversity, ecological complexity and evolutionary dynamics of the pre-Ediacaran biota. □

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Marine geology

Sea-floor volcano erupts ideas

Joe Cann

Was there a huge volcanic eruption on the floor of the Pacific Ocean in 1965 that covered an area of 220 km^2 with 15 km^3 of basalt lava? Such an eruption would have been comparable to the giant Lakagigur eruption in Iceland in 1783, which erupted 12 km^3 of basalt, poisoned the island's grasslands, killed many of the animals and led to a severe famine[1]. Yet the Pacific eruption was not observed at the sea surface, and the evidence can at present be only circumstantial. But even before its formal publication, the possibility has raised a kaleidoscope of new ideas in areas ranging from plate tectonics through hydrothermal activity to climate change. The case is made by Macdonald, Haymon and Shor in a paper shortly to appear in *Geology*[2] and draws evidence from various sources.

The primary evidence for the eruption comes from a survey of an area of the crest of the East Pacific Rise at 8–9° S. The East Pacific Rise is the mid-ocean ridge that separates the Pacific plate from the Nazca plate. At these latitudes about 0.2 m of new oceanic lithosphere is created on average each year by sea-floor spreading, the fastest rate known anywhere on the global mid-ocean-ridge system. The survey was made with the SeaMARC II towed side-scan sonar vehicle which acoustically measures ocean depths over a swath 10 km wide, and also constructs a side-scan sonar map, on which the intensity of backscattered sound is plotted on each side of the ship's track to show fault scarps (intensely reflecting strips), fissures (parallel shadows and intense reflections) and more complex shapes of volcanic seamounts (see the recent review article[3] by Macdonald *et al.*).

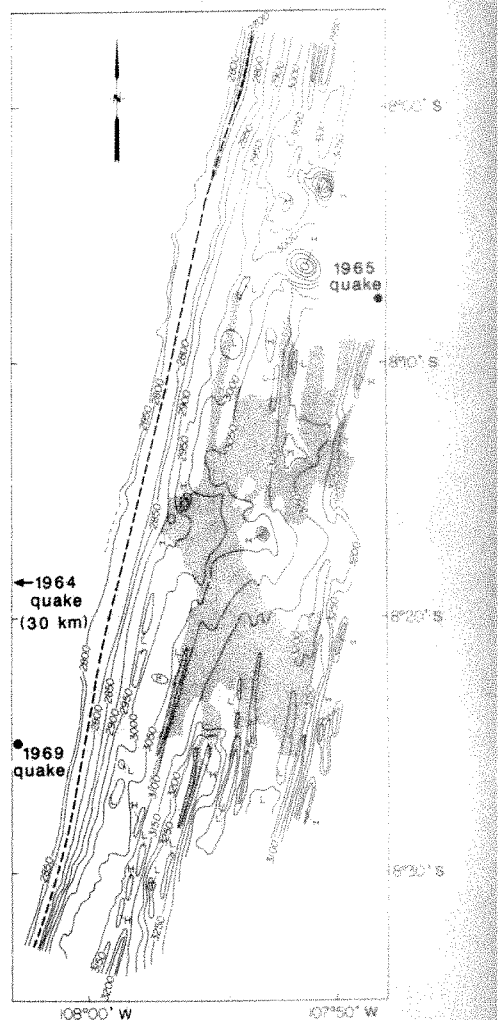
Between 8° 08′ S and 8° 28′ S, over a distance of 35 km, a few kilometres east of the spreading axis, marked here by a 1-km-wide shallow rift valley, appears an irregular area of high reflectivity (see figure). The bathymetry shows this area to be confined to topographic lows, to terminate against fault scarps and against a short chain of craters 3-km east of the rift valley. The plan shape is very similar to that of lava flows on the sea floor and on land, and there can be little doubt that this area is a lava flow emerging from the chain of craters. The thickness was calculated from where the flow abuts fault scarps, and the volume of 15 km^3 follows from the surface area.

How young is the flow? High reflectivity has been correlated in other places with young, glassy lava flows still not covered by sediment. A date in the 1960s is suggested by three earthquakes recorded in 1964, 1965 and 1969 from this general area. It is very unusual to record earthquakes at fast spreading ridges except from the transform faults that offset them and the nearest transform fault is 100-km south of this area.

Suppose the lava flow was erupted in the mid-1960s. Could it be related to a giant plume of ^{3}He-enriched sea water discovered in 1970–72 on the west side of the East Pacific Rise at 13° S by Lupton and Craig[4]? Magma from the Earth's mantle brings ^{3}He up as new lithosphere is created by sea-floor spreading, and can be emitted into the ocean by volcanic gases, or stripped from lavas by hydrothermal solutions. In such young magma, the ^{3}He/^{4}He ratio is 100 times the atmospheric ratio, and inputs to the deep oceans can be traced thousands of kilometres from their source.

The plume was originally ascribed to very active hydrothermal venting at the ridge crest at 13° S carried west by deep ocean currents. Now, Lupton and Macdonald[5] argue that the plume could result from the volcanic eruption at 8° S, and that deep ocean currents would first have carried it south and then west at 13° S. This raises the first of the new ideas that have emerged from this work. The ^{3}He plume has been used to estimate the steady-state hydrothermal flux into the oceans and to suggest that an especially active hydrothermal vent field lies at 13° S on the East Pacific Rise. If it has a volcanic source, that would alter the arguments drastically, and would require a re-examination of the whole question of hydrothermal flux of heat and elements. This idea is readily tested: if the plume was volcanic in origin, it should no longer be present, as eruptions of this kind must be very rare events.

The volume of magma erupted each year along the global mid-ocean-ridge system is about 3 km^3 (assuming the thickness of ocean crust lavas is 1 km, as indicated by ocean drilling and ophiolite stratigraphy). The 8° S eruption would thus account for 5 years' world production of lava. Perhaps more relevant is the lava production at the 75-km-long spreading segment on which the eruption happened.



Map of the East Pacific Rise around 8° S, showing the extent of the lava flow (stippling) and sites of earthquakes in the 1960s. Contours, 50 m; broken line, spreading axis. (From ref. 2; courtesy of K.C. Macdonald.)

At the local spreading rate of 0.2 km yr^{-1}, the flow represents 1,000 years' supply of lava erupted at one place and time.

There are two ways in which new sea floor is created: by magmatic intrusion and extrusion to form new crust; and by stretching of pre-existing crust. When the latter happens on a large scale, as it does in the North Atlantic, the magnetic-anomaly pattern is degraded and outcrops of lower-oceanic crust are found at the sea floor. Such effects would be seen on the East Pacific Rise if eruptions occurred only once every 1,000 yr and each covered less than 10 per cent of the sea floor accessible to major eruptions. In 10,000 yr, about 2 km of new sea floor forms at these fast spreading rates, and most of this would have to be produced by crustal stretching if eruptions were episodic. In fact, the East Pacific Rise has a continuous lava carapace, outcrops are confined to fracture zones, and the magnetic anomaly pattern contains much fine detail. Probably no more than 10 per cent of lava can be erupted in major flows if both of these conditions are to be maintained, which would mean that the world-wide recurrent interval of major flows must be more than 50 yr, perhaps several hundred years. Indeed, the Lakagigur eruption in Iceland may have been the most recent eruption of this kind preceding the Pacific one.

Macdonald *et al.* discuss the effect of the eruption on the axial magma chamber that has been demonstrated to underlie the crest of the East Pacific Rise elsewhere. They estimate that the flow represents about 10 per cent of the volume of the magma chamber, but in doing so, I believe, they overestimate the size of the chamber: the flow may represent as much as half its volume. In either case, the eruption should have resulted in a significant deflation of the ridge crest at least in the local area of the eruptive craters. Yet the ridge-crest morphology is described as inflated and appears so on the map. If this is the case, then the eruption (which emerged apparently not from the crest of the ridge but from the base of the axial high 2–3 km from the spreading axis) must have resulted from some extra inflation, perhaps from the sudden injection of a large volume of new magma into an already inflated magma chamber.

This presents another fascinating aspect of the problem. Recently McKenzie and colleagues[6] succeeded in convincing many volcanologists that magma must dribble out of the mantle as soon as it forms, and that large bodies of magma cannot form within the viscous asthenosphere which underlies the mantle. If here we have a spectacular counter-example, suggesting a sudden input of several cubic kilometres at once into a magma chamber at one place, that would have a profound effect on this line of thought.

By coincidence other apparently young and massive sea-floor lava flows have been discovered recently[7]. But these are not at spreading plate boundaries. Instead they cover large areas of the deep ocean floor around the Hawaiian islands. The flows were discovered during a systematic survey of the US exclusive economic zone with the British side-scan sonar GLORIA. In one place the flows cover 150,000 km^2, and fresh glassy basalt has been recovered, showing that the flows are young. It is not clear whether these flows are related to the island volcanoes (they are found in deep oceans 1–300-km offshore) or to the magmatism of plate boundaries (they are in the centre of the Pacific plate). Whatever the case, they clearly represent another submarine volcanic enigma.

The discovery of these apparently young, large lava flows has led Shaw and Moore[8] to speculate that large sea-floor eruptions might be a trigger for the El Niño climatic fluctuations of the equatorial Pacific. They suggest that the amount of heat released from a young lava flow of this size would be enough to produce sufficient warming of the sea surface layers to generate climatic fluctuations. This, I think, is the easiest of the ideas spawned by the East Pacific eruption to test. Not only would the frequency of such eruptions be too low to generate the observed frequency of El Niño, but Shaw and Moore entirely neglect the problem of getting the heat to the surface. For a plume of hot water to penetrate the stratification of the deep ocean, and then the thermocline, in the well-stratified equatorial Pacific, would apparently require a buoyancy flux of far greater magnitude than could be provided by even a large eruption. Surely that trail must be a dead end.

But if a single observation has generated so many interesting sparks even before its formal publication, there must be fair mileage in it yet. The next step must be to go and examine the lava flow, to collect samples for analysis, to determine its helium-isotope content and to determine its age (which will not be easy). The helium plume must be revisited as a matter of urgency to test that side of the story. Then, of course, it might be a good idea to have another look at the more accessible Lakagigur flow. How does that fit into the scheme of global and Icelandic magma plumbing? How is that related to processes in the mantle and to the history of Icelandic volcanism? We'll see. □

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Daedalus

Time is money

QUEUEING, especially in long institutional queues, implies that while the time of the institution is valuable, that of the queuer is worthless. This outlook probably reaches its high point in the Soviet Union, where you can queue indefinitely for a permit to join another queue; but high marks must also go to the packed waiting rooms and overbooked appointments systems of Britain's much-loved National Health Service.

Daedalus now presents a practical solution. The DREADCO Op-time-miser® is a sort of combined register, computer and vending machine. Installed in (say) a hospital waiting room, it accepts details of each patient to transmit ahead to the doctors. It also notes his time of arrival, and requests him to state the value of his time in pounds per hour. It already has this information for those already present: so it can calculate a 'break-even waiting time' for the group as a whole. Those who value their time highly are assigned a shorter waiting time than this, and pay for the time they save; those who value it modestly wait longer, and are paid their stated rate for the added inconvenience. The 'break-even time' is calculated so that the money paid in by the short-stayers is just sufficient to recompense the long-stayers. The group transaction always breaks even.

Thus the usual dreary waiting for broken appointments is eliminated. Patients just turn up out of the blue and immediately receive an estimate of their waiting time and its likely cost or reimbursement. This time may change as later arrivals modify the optimization; but each queuer knows how much he will be paid for added inconvenience, or charged for speedier service. The rich, as ever, can buy their way to the head of the queue; but the poor are not aggrieved, as they get the money. Nobody will put an absurdly high value on his time; for that increases the chance that he will have to pay out at that rate. But a deposit may have to be charged, to deter healthy layabouts who set a very low value on their time so as to sit endlessly in the warm waiting room at a slowly growing profit.

The Op-time-miser will revolutionize public life. In hospitals, passport offices and unemployment bureaux, wherever an inflexible bureaucracy confronts a resentful public, it will rationalize and smooth their dealings. Daedalus also plans to sell its software to all those booking, repair and installation services that have to juggle unpredictable customer requests into a queue. It will compensate for the sort of maddening delays and broken promises that gave rise to this announcement in the 'Births' column of a British national newspaper: "To Mr and Mrs Smith. Gratefully, after seven years. A telephone". David Jones

Ambiguities in stereopsis

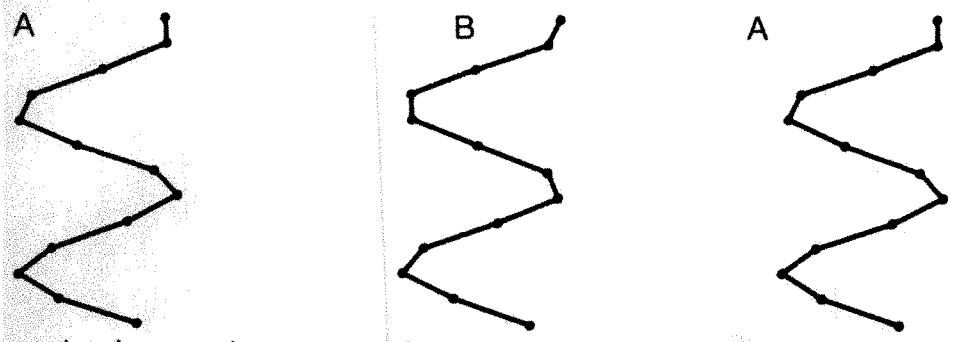
SIR—Stereo-pairs, usually of molecules, are regularly printed in many journals and add a useful third dimension to the traditionally two-dimensional printed page. I wonder, however, how many readers actually see stereo-pairs in three dimensions with correct orientation. An informal poll of my associates reveals that almost no one keeps a stereoscope handy; only about half knew that stereo-pairs could be viewed in three dimensions with the naked eye (direct stereopsis); about a quarter can use direct stereopsis; and only one person knows that a stereo-pair is ambiguous; it does not distinguish between right-handed and left-handed three-dimensional structures.

The ambiguity arises because a stereo-pair can be viewed by two methods[1]. Conventional stereoscopes use the uncrossed visual axes method — lenses, prisms or mirrors cause the right and left eyes to view the right and left members of the pair, respectively. But stereoscopes that use the crossed visual axes method can also be constructed. In these, the right eye views the left member of the pair, and *vice versa*. The depth dimension reverses with a change in viewing method, and the same stereo-pair that gives a right-handed three-dimensional image with one method gives a left-handed image with the other. In effect, the right and left members of the pair are exchanged in position.

Either method can also be used in direct stereopsis[2,3], but the crossed axes method seems easier to learn[2,4]. Therefore, most people who use direct stereopsis will see a reversed image of a printed stereo-pair because the images are usually arranged for conventional stereoscopes, which use the uncrossed visual axes method.

A stereo-triplet (suggested by Bruce Nicklas) consisting of equally spaced members A–B–A, illustrates the reversal (see figure). If A on the right is covered, A and B on the left form a stereo-pair. If A on the left is covered, B and A on the right form the same stereo-pair but with the members exchanged in position. For a given viewing method, the two pairs yield three-dimensional images with reversed depth and handedness. Surprisingly, viewing either pair with all members of the triplet uncovered yields two three-dimensional images side by side, reversed in depth and handedness. In general, a row of n members yields $n-1$ three-dimensional images with a two-dimensional image at each end of the row. This pattern occurs because fusion of the retinal images of any pair shifts the retinal images of the entire row out of register by one member on each retina.

Stereo-pairs as illustrations of three-dimensional objects are now easy to produce with microcomputers and three-dimensional drawing programs. Authors should make such illustrations unambiguous by stating whether they are arranged for viewing with crossed or uncrossed visual axes.



A stereo-triplet of a helical structure. Stereopsis of any two adjacent members yields two three-dimensional images with reversed depth and handedness. With crossed visual axes, the image on the right is right-handed, like a conventional screw thread. With uncrossed axes, it is left-handed.

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Earthquake records

SIR—Roger Bilham[1] has made a powerful case for a global approach to the long-term forecasting of earthquakes and, more specifically, for the use of the techniques of space geodesy to detect premonitory ground deformation. Although strain is a fundamental measure of the energy released by earthquakes, it may not always be recognizable at the surface using geodetic techniques. Therefore, we need also to understand the historic and prehistoric record of earthquakes in much greater detail than we do at present.

Long and well-dated earthquake chronologies[2] bring other benefits: they may reveal seismic zones that are temporarily inactive[3] and they are bound to throw light on the mechanisms responsible for the earthquakes[4].

In any case, the geodetic approach requires information on the strain release of a complete earthquake cycle at the site in question. In other words, it will not pay off until the next earthquake. As Bilham observes, that may be decades or centuries in the future: one of the primary tasks of palaeoseismology is to seek out any periodicity (or its lack) in the local occurrence of earthquakes of different sizes.

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Island occupation

SIR—In his recent News and Views article[1] on the initial human occupation of the islands of the south-west Pacific, Diamond asked the rhetorical question of whether Pleistocene colonists had managed to reach "only the largest visible targets, leaving Bismarck outliers to be discovered tens of thousands of years later by Lapita explorers?" The answer, as far as a south-eastward thrust down the islands of the Solomon chain is concerned, is no.

Wickler and Spriggs last year investigated[2] Kilu cave on Buka Island, just north of Bougainville Island in the northern Solomons (see map in ref. 1), and found late Pleistocene occupation deposits spanning the period from 20 to 28 kyr ago. These levels contain evidence for the exploitation of marine gastropods, fish and, judging from residue analysis of starch grains on some stone flakes, vegetable tubers. Being an oceanic island, the land fauna is depauperate but included some endemic murids which became extinct, probably because of human impact. This pattern of exploitation is similar to that shown[3] in the Pleistocene occupation levels at the coastal cave of Matenkupkum, New Ireland, 33 kyr ago.

Buka is 175 km away from New Ireland and cannot be seen from there. Between the two islands are the small Feni and Nissan groups, but a journey from New Ireland to Buka via the Feni group would still involve a sea crossing of at least 145 km; and a crossing using both Feni and Nissan groups would be not less than 70 km. Nissan is a coral atoll, and it is not

clear that it was even in existence at that time, as the sea level would have been about 45 m below its present level. The angular target for a voyage directly from New Ireland to the Buka–Bougainville group would have been 30°, and using the island stop-overs, some stages would have had targets as little as 8°.

Once on Buka, the crossings to most of the main islands of the northern Solomons would have been easy as far south as San Cristobal, 1,100 km away. The gap to Santa Cruz probably marked the boundary of Pleistocene occupation. Waisted axe-like tools similar to those dated to 40 kyr ago[4] at the Huon peninsula on the New Guinea coast opposite New Britain have been found on several of the islands of the group. The diversity of human genetics on Bougainville indicates that the islands must have been occupied at least 10 kyr ago[5], a figure that until recently seemed impossible. The colonization of the large islands of the Bismarck and northern Solomons chains occurred soon after that of the Australian–New Guinea continent itself[6]. A sea-crossing capacity, probably denied to the south-east Asian *Homo erectus* populations, was one of the hallmarks of modern man in the region, the events occurring at about the same time that modern *H. sapiens* groups colonized western Europe.

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Viral proliferating cell nuclear antigen

SIR—We have identified in the genome of the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcMNPV), a gene that encodes a protein similar to proliferating cell nuclear antigen (PCNA), also termed cyclin[1] or the polymerase-δ auxiliary protein[2,3], which is associated with DNA replication in mammalian cells.

The gene is the previously characterized[4] ETL, which is similar in size to rat PCNA and has 42 per cent amino-acid identity. Conserved sequences are distributed throughout the protein and very few insertions or deletions are required to generate the optimal match (see figure).

Evidence that this viral PCNA is involved in DNA replication is provided by the mutant of AcMNPV, vETLβgal[4], which contains the *Escherichia coli* β-galactosidase gene (*lacZ*) fused to the amino-terminal 165 amino acids of the viral PCNA. This mutant is viable in both insect cell cultures and in perorally infected larvae, but all late viral gene expression, which requires DNA replication, is 4–6 hours later in vETLβgal-infected cells than in wild-type virus-infected cells[4].

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MFEAEFKTGAVLKRLVETPKDLLPHATFDCDNRGVSMQVMDTSHVALVSLQLHAEGFKKY
****  |  *  |**|||* |***|  *  *    ** |* ** *** ** * *| *** *
MFEARLIQGSILKKVLEALKDLINEACWDISSGGVNLQSMDSSHVSLVQLTLRSEGFDTY

RCDRNVPLNVSINSLSKIVKCVNERSSVLMKAEDQGDVMAFVFN--NDNRICTYTLKLMC
******| | *  | *|***|**       | ||***  *  |*|**    *|  ||   *  |***
RCDRNLAMGVNLTSMSKILKCAGNEDIITLRAEDNADTLALVFEAPNQEKVSDYEMKLMD

                                                    ↓
IDVEHLGIPDSDYDCVVHMSSVEFAQVCKDMTQFDHDIIVSCSKKGLQFRANGDIGSADV
|***|****| |* *** * * *** |*|*| |     |||** * *| * * *||*  ||
LDVEQLGIPEQEYSCVVKMPSGEFARICRDLSHIGDAVVISCAKDGVKFSASGELGNGNI

QM-----SADNENFSV-LKAKQTVTHTFAGDYLCHFAKAAPLAPTVTIYMSEELPFKLEYC
  |       * *   * |   | *  ***  **  * ** ** ****| ** ||*   |**
KLSQTSNVDKEEEAVSIEMNEPVQLTFALRYLNFFTKATPLSPTVTLSMSADVPLVVEYK

IKDVGVLACFLAPKIVNNDEEIF-
* *|* *   *****   ***
IADMGHLKYYLAPKI--EDEEGS-
```

Comparison of the amino-acid sequence of ETL[4] (top lines) with rat PCNA[9]. Asterisks, identical residues; lines, substitutions which occur at greater than average frequency among related proteins. Vertical arrow, point at which the ETL sequence is disrupted in vETLβgal.

We have recently confirmed that vETLβgal DNA replication is delayed. Because the mutant virus is viable, however, viral PCNA does not appear to be absolutely required for DNA replication, at least in proliferating cell cultures. Perhaps, viral PCNA is essential for virus replication in nonproliferating, quiescent cells.

In addition to inducing the host DNA polymerase-α[5,6], baculoviruses encode a DNA polymerase[7] which is sensitive to aphidicolin[5,8] and has an associated 3′-to-5′ exonuclease[8], properties shared with DNA polymerases-δ. The induction of these replication-related enzymes may contribute to the ability of AcMNPV to establish an S-phase environment and thereby replicate efficiently in proliferating and quiescent host tissues.

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Dystrophin digest

SIR—We have recently reported[1] the localization of a protein of relative molecular mass 210,000 (210K) sharing an antigenic determinant with the 400K dystrophin on the surface membrane of normal human skeletal muscle fibres. Hoffman *et al.*[2] have since suggested that the 210K protein may be a degradation product of dystrophin, and our own data now support this suggestion.

In previous experiments, we found the 210K protein but not the 400K protein in crude and DEAE-cellulose purified low-ionic-strength extracts of skeletal muscle. But when we isolated the protein samples from frozen (−80 °C) or fresh muscle specimens by direct solubilization of tissues in a gel-loading buffer, containing 10 per cent SDS as described by Hoffman *et al.*[3], instead of the 1 per cent SDS used in our previous report, an immunoreactive 400K band could be recovered with our antibody. Further, we found that the antibodies produced by Kunkel's group (30K, 60K) crossreacted with the 210K protein in our low-ionic-strength extract of skeletal muscle.

We now believe that the 210K protein is probably a part of 400K dystrophin, and that native dystrophin, which is extremely susceptible to proteolysis *in vitro*[4], can easily be digested during the preparation process. The digestion may occur at fixed break point(s), because the 210K protein is always observed[1] as a sharp, single band. Thus, the 210K protein presumably represents a stable portion of the 400K dystrophin.

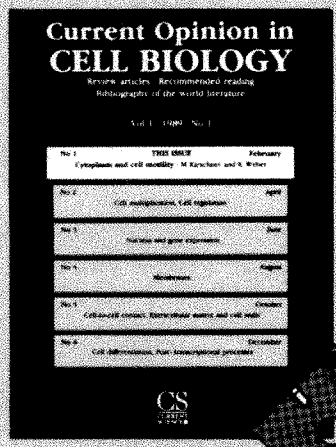
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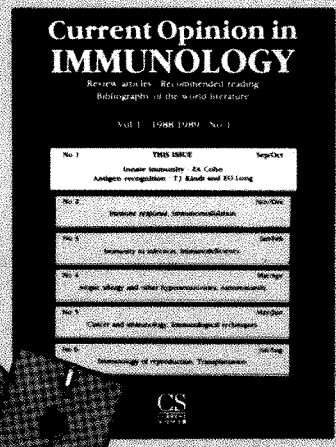
Division of Neuromuscular Research, National Institute of Neuroscience, NCNP, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187, Japan

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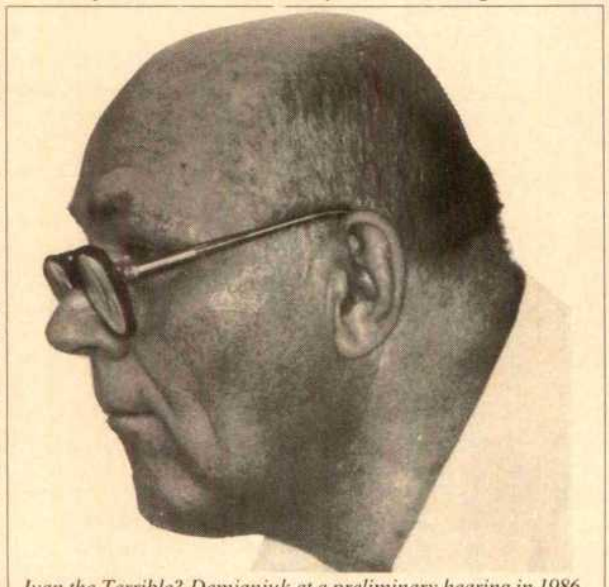
The face of justice?

John C. Marshall

Identifying Ivan: A Case Study in Legal Psychology. By Willem A. Wagenaar. *Harvester–Wheatsheaf/Harvard University Press: 1988/1989. Pp. 187. £13.95, $20.*

ALL trials are, or should be, 'show trials'. In accordance with the axiom "Justice must not only be done, but must be *seen* to be done", they should show as unambiguously as possible whether the accused is innocent or guilty as charged. Nazi and Stalinist show trials were obnoxious not because they were spectacles, but because those found guilty were so manifestly innocent.

Last April, a Jerusalem court sentenced John Demjanjuk to death after a trial explicitly stage-managed to remind Israel's youth of the Jewish past in Europe; his appeal is pending. The crime is not at issue. A Ukrainian guard at Treblinka death camp was, in part, responsible for the murder of 850,000 Jews in the gas chambers. The court decided that this man, known to the prisoners as Ivan the Terrible, was John Demjanjuk, a factory worker from Cleveland, Ohio, who had been extradited from the United States to stand trial in Israel. Demjanjuk's defence was simple in principle, although just how complex in fact is revealed by Willem Wagenaar's monograph *Identifying Ivan: A Case Study in Legal Psychology*. The defence was mistaken identity — that Demjanjuk was not 'Ivan'.



Ivan the Terrible? Demjanjuk at a preliminary hearing in 1986.

Willem Wagenaar is a distinguished Dutch scientist who holds the chair of Experimental Psychology at the University of Leiden. His areas of professional competence include the study of memory and perception. He is an acknowledged expert on identification procedures and eyewitness testimony, and it is in this capacity that he agreed to act in the defence of John Demjanjuk. Other equally qualified psychologists had declined to so testify, fearful apparently of the reactions of friends and colleagues. Wagenaar's decision (and the whole tone of his book) is evidence of his intellectual and moral strength; both his character and the technical nature of his testimony have subsequently been vilified and distorted by many who ought to know better.

Quite apart from the specific discussion of Demjanjuk's case, the book is a vivid tutorial on the reliability (or otherwise) of facial identification and the rules for the conduct and interpretation of identity tests. These are areas to which experimental psychologists have made substantial contributions and have succeeded in elucidating a wide range of perceptual, memorial, cognitive and social factors that affect the validity of identification judgements; studies of this topic deliver highly pertinent information to counter those who argue that empirical psychology is conceptually vacuous and socially useless.

Wagenaar summarizes the relevant experimental evidence superbly and provides an acute analysis of its strengths and weaknesses; he falls into neither the trap of assuming that the expert is always right, nor that of dismissing scientific work because the knowledge so obtained is itself limited and fallible. Furthermore, he convincingly relates such laboratory studies to well-documented cases where flaws in line-up and show-up procedures are known (in retrospect) to have resulted in a miscarriage of justice. A number of Wagenaar's conclusions were prefigured in the document prepared by a British advisory committee, chaired by Lord Devlin (1976). Wagenaar pays due tribute to the Devlin Report, although the pace of research has now materially added to the body of information available. His monograph should accordingly be obligatory reading for all judges and lawyers — or better, for all citizens.

The central section of *Identifying Ivan* is a description and justification of 50 procedural rules for the fair conduct of identity parades. They include such seemingly reasonable requirements as (Rule 17): "Witnesses should, before participation in an identification test, be requested to provide a verbal description of the criminal, as they remember him. These descriptions should be included in the reports of the subsequent identity test". And (Rule 21): "The instruction to the witnesses should stress that the wanted person is possibly not in the parade or photospread, and that therefore a positive response should be made only when the witness is certain of recognizing that person".

It is Wagenaar's contention that of the 46 rules applicable to Demjanjuk's case, 28 were directly broken and another nine violated by implication from earlier flaws. Similar procedural errors were made, Wagenaar claims, at the Cleveland trial in 1981 when Demjanjuk's US citizenship was revoked. It is true, as Wagenaar readily concedes, that not all of these rules are accepted as an international standard. Nonetheless, the least that can be said on the basis of his quietly rational documentation is that justice was not *seen* to be done. Such violations could not, of course, occur in Britain. But at a time when the British government is attempting (with some success) to turn the state into an appendage of MI5, we should perhaps be aware of the problem.

Professor Wagenaar does not pretend to the wisdom of Solomon, and he is far too intelligent a man to dispute the verdict of the Jerusalem court. That decision was based upon more evidence than is available to an expert witness for the defence. As Wagenaar states: "The process of translating prior odds into posterior odds is influenced by the experts' likelihoods, but not determined by them". For the reader who wants to know the Truth, Wagenaar "can only refer to the court's verdict". It is, he writes, "the best answer we have".

I do, however, take away from his book one firm conclusion. We can all agree that no legal process will be perfect (at least until the Messiah comes); but there is, to my mind, a strong case to be made that *all* expert testimony should be presented not by 'experts' for the prosecution or the defence, but rather by 'friends of the court'. Until that day, Wagenaar might like to recall an old Yiddish story: The rabbi's wife was convinced that her servant girl was stealing from her. She told the rabbi that she intended to take the girl to court. When the rabbi said that he too would go, the rebbetzin informed him that she knew the law as well as he did and could successfully prosecute on her own. "Ah, yes", said the rabbi, "but who save me will dare to testify in your servant's defence?". □

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Fields in chaos

Ian Percival

Nonlinear Physics: From the Pendulum to Turbulence and Chaos. By R.Z. Sagdeev, D.A. Usikov and G.M. Zaslavsky. *Harwood: 1988. Pp.675. Hbk $98, £55; pbk $39, £21. Six accompanying diskettes $77, £43.*

THE main authors of this book, Sagdeev and Zaslavsky, have made notable contributions to nonlinear physics. A few years ago Zaslavsky moved from Krasnoyarsk to work with Sagdeev at the Space Institute in Moscow, and the book is one result of their collaboration.

It is an ambitious venture, written for the physicist who wants to use nonlinear dynamics, and includes many analytic and numerical methods and results that have been developed in the past few decades. Its particular strength is the recognition of the important role of chaotic dynamics in many fields, particularly plasma physics. The emphasis is strongly on hamiltonian systems and on applications.

The authors begin with the more traditional methods of hamiltonian dynamics, but from a modern point of view. Then follows a section of special methods; these are largely developments of Poincaré's ideas as tools for applications, but include the KAM theorem. The next hundred pages deal with various aspects of chaos, most of it hamiltonian. There is a short chapter reviewing the principles — which derive largely from the work of mathematicians — and then a long one giving a detailed description of some of the complicated and subtle properties of hamiltonian chaos; most of these have been discovered by numerical experiment using computers, but backed by theory. The next chapter, on kinetic theory, concentrates on those aspects of transport which are of importance in plasma physics.

Part 2, on nonlinear waves, starts with stationary waves, including normal and collision-free shocks, ion-acoustic and magnetosonic waves; the connection made with the theory of particle motion is helpful here. Exactly integrable wave equations are introduced in the context of their applications, and are then treated in more detail later. There follows a description of the hamiltonian formulation, including resonant interaction, resonances and interaction of phonons, and two long chapters on chaos in wave fields and strong turbulence. Strong turbulence is introduced through the Lorenz model, and the distinction between temporal and space-time chaos.

Part 3 gives examples, and is really a collection of bits and pieces that might not have fitted naturally into earlier sections. The first and best is on the motion of particles in wave fields, to which the authors have made important recent contributions and which are illustrated with strikingly beautiful computer-produced colour plates at the end of the book. The second is on billiards, but is too brief to do justice to the subject. The connection with the ray theory of wave optics is considered, but there is no mention of the work of Keller, Maslov, Gutzwiller, Berry and many others on the connection between rays and waves in general, and billiards and cavities in particular.

A later section deals with nonlinear chains, especially with the mathematical equivalence of the equilibrium of incommensurate structures and the dynamics of certain maps. Here, however, there is no description of the Frenkel–Kontorova model, or of Aubry's classic work on its correspondence with the standard map. There are also chapters on applications to nonlinear optics, and to the perturbed Kepler problem (with applications to astronomy and to the excitation of Rydberg atoms). Each provides enough to whet the appetite, but falls far short of being a modern review of the field.

The fourth and final part of the book, by Usikov, is the most unusual. It provides a detailed guide to the programming methods necessary to explore some of the dynamical systems with the aid of an IBM-compatible microcomputer. The detailed listing is not provided, though disks can be obtained at an extra cost.

Part of the problem in writing a book of this kind is that the detailed theory is presented analytically, with some help from geometry, whereas many of the advances are made with the aid of computer experiments, which cannot be put directly into print. So the normal method of presentation is misleading. The final part of the book tries to redress this situation. The 'colour supplement' of orbit simulations is valuable, and will encourage readers to experiment for themselves. But it is not so clear that the computer programs will be so useful, and there is a strong risk that they are becoming outdated — nowadays people use mice!

It is said that Enrico Fermi's use of English was often wrong, but that it was best not to correct it because the errors were minor and had such charm that it was a pity to remove them. Much the same can be said of this book — for example "multiple period birth bifurcations" (p.199) looks more like a term from gynaecology than nonlinear dynamics; but we know what the authors mean. More serious is the use of the words "chaos", "stochasticity" and "turbulence" without the distinctions of modern usage, and the use of "soliton" for interacting solitary waves.

My impression is that the first three parts of the book were written by two busy and productive scientists, who naturally concentrated on the areas to which they have made research contributions. They did not, it seems, have the time to bring all the chapters up to date, nor to provide the polish, revision, indexing, references and cross-references which would have made this a much better volume. For example, although the references provide a guide to some important Soviet literature, they are by no means comprehensive.

Nonetheless this is a valuable contribution to the literature on nonlinear physics. And unlike Zaslavsky's earlier book *Chaos in Dynamical Systems*, which was terribly overpriced, it is very good value for money, especially in paperback. □



Living totem pole — "Young Condor on Post, Steamboat in Background, Jenning's Lodge, Oregon, 1906", a high-quality photograph on a 5 × 7-inch glass plate by W. L. Finley and H. Bohlman. The picture is taken from a chapter on printing old negatives in Alfred A. Blaker's Handbook for Scientific Photography, *2nd edn, just published by Focal Press (an imprint of Butterworth), price $45, £39.95.*

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Issues of organic origins

James P. Ferris

The Emergence of Life: Darwinian Evolution from the Inside. By Sidney Fox. *Basic Books: 1988. Pp. 208. $17.95.*

SIDNEY FOX, a pioneer experimentalist on the origins of life, has vigorously promoted research into the subject and his particular approach to it since the 1950s. His work has prompted debate and controversy, and has sparked interest in the field. In *The Emergence of Life*, he summarizes his findings and conclusions for those with a basic understanding of high-school biology. Readers, however, should take to heart the warning to be found in the preface: "I feel comfortable with a frankly acknowledged bias derived from experiments and experience". It appears that bias has indeed played a strong role in the development of his ideas. In this review I will examine in detail two of the basic precepts of Fox's theory which I believe are built on distinctly wobbly experimental foundations.

Polymers

The first is that ordered polymers are formed by the thermal polymerization of amino acids. Fox's view that protein was the basis for the first life stems from his observation that polymers are formed by the dry-phase heating of amino-acid mixtures which contain an excess of either glutamic acid, aspartic acid or lysine up to 80°C. These polymers are not linked simply by peptide bonds; rather, many of the bonds (33–100 per cent in the case of polymers formed with excess aspartic acid) arise from chemical interaction with the carboxyl or amino groups that are not involved in normal peptide bond formation. Fox clearly feels that they are protein-like and justifies this in part because *Chemical Abstracts* indexes them as "thermal proteins". But indexing is one thing, experimental evidence is another.

Is the thermal polymerization of amino acids on the early Earth a plausible idea? It is not beyond the realm of possibility, but other questions remain. For example, how did the excess of acidic or basic amino acids accumulate? Aspartic and glutamic acids are among the more abundant amino acids formed in experiments simulating the primitive Earth. But glycine and alanine are usually formed in much larger amounts, while lysine is formed in trace amounts only. So it is difficult to understand how the basic thermal polymers came into being. Other organic substances may have inhibited the polymerization process because it is highly unlikely that one would find an area on the primitive Earth where only amino acids concentrated. For example, the effect of carboxylic acids and amines on the thermal polymerization of amino acids merits investigation, because they may have accumulated together with acidic or basic amino acids.

It may be possible to overlook problems with the polymerization thesis if Fox's proposal that there are internal limitations on the structures of these thermal polymers is correct. The proposed internal ordering is the basis for an even more controversial assertion — that molecular memory of the type provided by DNA in contemporary life was not essential in the earliest life.

One experiment cited by Fox involved the copolymerization of a 1–2–1 mixture of glutamic-acid–glycine–tyrosine where only two tyrosine-containing tripeptides were isolated from the reaction mixture (higher molecular weight oligomers were also formed). These tripeptides have the composition pyroglu–gly–tyr and pyroglu–tyr–gly. Fox claims that this result is evidence of internal ordering, because there are 19 different ways in which three amino acids containing one or more tyrosines could be linked, yet only two were observed. This reasoning does not stand up to more detailed scrutiny. Polymers form in the presence of glutamic acid because it cyclizes rapidly to pyroglutamic acid first, and then amide bonds are formed with the carboxylic acid or imide groups of the pyroglutamic acid. Consequently, only a glutamic acid residue will be on the amino terminus of the chain. In reality, this is a system where the location of glutamic acid is fixed so only three tyrosine-containing dipeptides will be formed. In the experiment, the molar amount of glycine was initially twice that of tyrosine, and because tyrosine decomposes more readily than glycine under thermal polymerization conditions it is hardly surprising that the trimer containing two tyrosines was not detected among the reaction products. Thus the observation of only two tripeptides is consistent with the known chemistry of the reactants used and cannot be generalized to more complicated mixtures of amino acids.

The complexity of the reaction product mixture formed from 18 amino acids was revealed when it was subjected to DEAE-cellulose chromatography after initial purification by dialysis (Dose, K. & Rauchfuss, H. in *Molecular Evolution, Prebiological and Biological* (eds Rohlfing, D.L. & Oparin, A.I.) 199–217; Plenum, 1972). Preparations which appeared to be homogeneous in gel electrophoresis could be split into more than seven fractions. Even then it was not established that any of the fractions were homogeneous. No self-ordering was observed in this fractionated material, and indeed Fox never reports unambiguous data for self-ordering in thermal polymers. For example, critical experiments to define self-ordering, such as nearest neighbour analysis or sequence specific enzymatic hydrolysis, are not presented.

Protocells

The second of Fox's precepts with which I disagree is that protocells are formed from thermal polymers. When thermal polymers are dissolved in a small amount of boiling water and the mixture is allowed to cool, the polymers (which are insoluble in cold water) precipitate out as spheres 0.5–7 μm in diameter. Fox refers to these particles as microspheres, protocells or primordial cells. Microscopic examination of the particles indicates that there is a dark boundary layer at their surface which looks like a membrane. The apparent presence of boundary material suggested to Fox that if these spheres formed on the primitive Earth, they may have encapsulated other organic material and this system eventually evolved into the first cell. Unfortunately, most of the microsphere material dissolves when the solution in which they form is diluted with water or buffer, while the dark material that looks like a membrane dissolves partially. Fox tested the permeability of this dark material to carbohydrates by forming the microspheres in the presence of solutions of glucose, fructose, starch or glycogen. The microspheres were washed with water and after four washes there was still glycogen or starch remaining in the fraction with the dark material, but fructose or glucose was completely removed. But Fox reports only a few experiments, so it is impossible to determine how much of the original encapsulated carbohydrate remained after washing the microspheres (Fox, S.W. *et al.* in *Physical Principles of Biological Membranes* (eds Snell, F. *et al.*) 417–432; Gordon & Breach, 1970).

Finally, Fox and his colleagues noted in the same article, "How much of this [bound carbohydrate] is attributable to the boundary is not known". Thus, whether the carbohydrate is bound to the dark material or whether it is really retained inside a cavity formed by the boundary material was not established. There are no reports of the encapsulation of authentic protein or nucleic acid within the microspheres. Microparticles (no boundary layer) are formed between lysine thermal polymers and nucleic acids as a result of the charge interaction between the protonated lysine and the phosphate anions of the nucleic acids. One would expect that nucleic acids would be retained inside the microspheres formed from acid thermal polymers if there was any sort of a membrane present, but such studies were not reported. Fox has little experimental support for the assertion that there is a lipid-like membrane at the surface of these microspheres

— it seems that no structural analyses of the presumed boundary material were carried out. The failure to retain thermal polymers, and the absence of data on the retention of protein and nucleic acids, leads me to the opinion that microspheres formed from thermal polymers could not have served to encapsulate the first life on the primitive Earth.

Because these fundamental proposals have such poor experimental support, I am forced to conclude that Fox's theory has little validity. Consequently, the other claims to be found in the book — on the significance of the budding of microspheres, their implied sexuality, the 'membrane' potentials and so on — are meaningless in the context of the origins of life. This is not to say that polypeptides did not have a role in the first life, or that there might have been some sequences that formed more readily than others, but it does not seem likely that proteins with specified sequences formed without some sort of a catalyst (code?) to direct their formation. Investigation of syntheses using conditions that are more selective than dry, thermal polymerization may shed some light on prebiotic peptide formation.

On the positive side, the book provides a lively account of Fox's ideas and of how they developed. Fox is a strong proponent of the view that the most useful insights for the understanding of the origin of life come from knowledge of the products of potentially prebiotic precursors and not from the examination of contemporary and ancient biochemical systems; that is a view that is gaining wider acceptance today. But most of the conclusions that he has drawn far exceed what is justified by the data. □

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Set and match

Ian Stewart

Fractals Everywhere. By Michael Barnsley. *Academic: 1988. Pp. 396. £27, $39.95.*

NOT too long ago there was only one book on fractals, Benoit Mandelbrot's *The Fractal Geometry of Nature*. Now there are several, but here we have the first genuine textbook on fractals *as* fractals (the admirable *Geometry of Fractal Sets*, by Ken Falconer, is too much a textbook on geometric measure theory to count here). Michael Barnsley has taught the material at the Georgia Institute of Technology, where his course is open to all students who have completed two years of calculus; the resulting volume could be used by final-year undergraduates or at postgraduate level.

Barnsley concentrates on the mathematics that underlies the theory of fractals: the only application discussed is to data-compression in computer graphics. He takes pains to convey the ideas behind the theorems, using diagrams as well as formal definitions to put across abstract concepts such as metric space, affine transformation and Hausdorff dimension.

The mathematical core of the book is the notion of a self-similar set, which is fundamental to the fractal point of view. A set is self-similar if there exists some finite system of transformations — or iterated function system — such that the images of the set under these transformations, when combined, yield the original set. In other words, the set can be split into a finite number of pieces, each of which has the same structure as the whole, but on a smaller scale. Every iterated function system possesses a unique invariant set — that is, a set which is self-similar under that system. This invariant set is in general a fractal, except for unusual choices of the function system.

One of the central results concerns the 'chaos game'. If an initial point is chosen, and transformations from the function system are applied to it at random to create new points, then with probability one the set of points so formed becomes closer and closer to the unique invariant set for the system. This forms the basis of a computer algorithm for drawing the invariant set. Barnsley deals in depth with the mathematical and dynamical features of the chaos game (including the fractal dimension of the invariant set), alongside its application to computer graphics. The ideas are also extended to a probabilistic version, in which the black-and-white decision 'point' or 'no point' is replaced by shading on a scale of grey. The mathematics behind the famous Mandelbrot set, and its associated Julia sets, is discussed in some detail.

This is a fetching book, with beautiful computer graphics (some in colour), as well as a host of more schematic diagrams, the aim of which is to clarify the abstract mathematics. The fractal ferns, in particular, are quite remarkable, and relate directly to the central ideas. The material is well chosen and hangs together coherently, and the writing conveys a proper sense of excitement. It is true that some of the proofs are tough going, and demand a certain mathematical maturity; but the book can be understood even if the occasional proof is set to one side for future investigation. If you want a genuine understanding of fractals as mathematics, and not just as attractive images, this is an excellent place from which to start. □

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Innervative ideas

Alun M. Davies

Body and Brain: A Trophic Theory of Neural Connections. By Dale Purves. *Harvard University Press: 1988. Pp.321. $35, £27.95.*

IT IS common knowledge that the brain controls the body. That the body has an organizing influence on the nervous system is not generally appreciated, yet maturation and growth in all but the simplest of organisms is accompanied by the continuous adaptation of neural connections to the body's changing needs. Purves takes this adaptation as the starting point for the exposition of a trophic theory of neural connectivity. The theory not only provides an important unifying principle in neural development, but has implications for several diverse areas of biological science.

In the context of the nervous system, the term trophic refers to the long-term interdependence between neurons and the cells they innervate. In development, these trophic interactions regulate the size of neuronal populations and have a part in modifying the extent and complexity of neural connections throughout life. Purves presents a comprehensive overview of the foundations of trophic theory from its origins in experimental embryology to our current understanding of its molecular basis.

Alongside the central theme of trophic interactions in development and growth, there is discussion, from an evolutionary standpoint, of the significance of the adaptability of the nervous system to changes in body size and form. The link between neural activity and the modification of connections is examined in terms of trophic theory, an issue which has a bearing on the molecular basis of learning. Finally, Purves evaluates the currently fashionable regressive theories of neural connectivity that are based on a direct analogy between neural development and natural selection. His arguments clearly expose the inadequacies of the naive view that the nervous system develops according to some abstract darwinian principle.

This is a scholarly, thoughtful and well-written book. It will be of value not only to neurobiologists but to anyone interested in basic biological issues. □

Alun M. Davies is Senior Lecturer in Anatomy at St George's Hospital Medical School, Tooting, London SW17 0RE, UK.

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Radiocarbon dating of the Shroud of Turin

P. E. Damon*, D. J. Donahue†, B. H. Gore*, A. L. Hatheway†, A. J. T. Jull*, T. W. Linick†, P. J. Sercel†, L. J. Toolin*, C. R. Bronk‡, E. T. Hall‡, R. E. M. Hedges‡, R. Housley‡, I. A. Law‡, C. Perry‡, G. Bonani§, S. Trumbore‖*, W. Woelfli§, J. C. Ambers¶, S. G. E. Bowman¶, M. N. Leese¶ & M. S. Tite¶

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Very small samples from the Shroud of Turin have been dated by accelerator mass spectrometry in laboratories at Arizona, Oxford and Zurich. As controls, three samples whose ages had been determined independently were also dated. The results provide conclusive evidence that the linen of the Shroud of Turin is mediaeval.

THE Shroud of Turin, which many people believe was used to wrap Christ's body, bears detailed front and back images of a man who appears to have suffered whipping and crucifixion. It was first displayed at Lirey in France in the 1350s and subsequently passed into the hands of the Dukes of Savoy. After many journeys the shroud was finally brought to Turin in 1578 where, in 1694, it was placed in the Royal Chapel of Turin Cathedral in a specially designed shrine.

Photography of the shroud by Secondo Pia in 1898 indicated that the image resembled a photographic 'negative' and represents the first modern study. Subsequently the shroud was made available for scientific examination, first in 1969 and 1973 by a committee appointed by Cardinal Michele Pellegrino[1] and then again in 1978 by the Shroud of Turin Research Project (STURP)[2]. Even for the first investigation, there was a possibility of using radiocarbon dating to determine the age of the linen from which the shroud was woven. The size of the sample then required, however, was ~500 cm^2, which would clearly have resulted in an unacceptable amount of damage, and it was not until the development in the 1970s of small gas-counters and accelerator-mass-spectrometry techniques (AMS), requiring samples of only a few square centimetres, that radiocarbon dating of the shroud became a real possibility.

To confirm the feasibility of dating the shroud by these methods an intercomparison, involving four AMS and two small gas-counter radiocarbon laboratories and the dating of three known-age textile samples, was coordinated by the British Museum in 1983. The results of this intercomparison are reported and discussed by Burleigh *et al.*[3].

Following this intercomparison, a meeting was held in Turin in September–October 1986 at which seven radiocarbon laboratories (five AMS and two small gas-counter) recommended a protocol for dating the shroud. In October 1987, the offers from three AMS laboratories (Arizona, Oxford and Zurich) were selected by the Archbishop of Turin, Pontifical Custodian of the shroud, acting on instructions from the Holy See, owner of the shroud. At the same time, the British Museum was invited to help in the certification of the samples provided and in the statistical analysis of the results. The procedures for taking the samples and treating the results were discussed by representatives of the three chosen laboratories at a meeting at the British Museum in January 1988 and their recommendations[4] were subsequently approved by the Archbishop of Turin.

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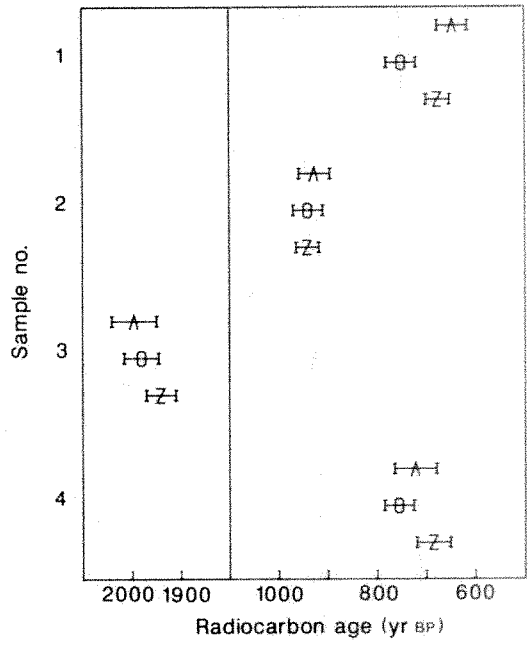


Fig. 1 Mean radiocarbon dates, with $\pm 1\sigma$ errors, of the Shroud of Turin and control samples, as supplied by the three laboratories (A, Arizona; O, Oxford; Z, Zurich) (See also Table 2.) The shroud is sample 1, and the three controls are samples 2–4. Note the break in age scale. Ages are given in yr BP (years before 1950). The age of the shroud is obtained as AD 1260–1390, with at least 95% confidence.

Removal of samples from the shroud

The sampling of the shroud took place in the Sacristy at Turin Cathedral on the morning of 21 April 1988. Among those present when the sample was cut from the shroud were Cardinal Anastasio Ballestrero (Archbishop of Turin), Professor L. Gonella (Department of Physics, Turin Polytechnic and the Archbishop's scientific adviser), two textile experts (Professor F. Testore of Department of Materials Science, Turin Polytechnic and G. Vial of Musée des Tissues and Centre International d'Étude des Textiles Anciens in Lyon), Dr M. S. Tite of the British Museum, representatives of the three radiocarbon-dating laboratories (Professor P. E. Damon, Professor D. J. Donahue, Professor E. T. Hall, Dr R. E. M. Hedges and Professor W. Woelfli) and G. Riggi, who removed the sample from the shroud.

The shroud was separated from the backing cloth along its bottom left-hand edge and a strip (~10 mm × 70 mm) was cut from just above the place where a sample was previously removed in 1973 for examination. The strip came from a single site on the main body of the shroud away from any patches or charred areas. Three samples, each ~50 mg in weight, were prepared from this strip. The samples were then taken to the adjacent Sala Capitolare where they were wrapped in aluminium foil and subsequently sealed inside numbered stainless-steel containers by the Archbishop of Turin and Dr Tite. Samples weighing 50 mg from two of the three controls were similarly packaged. The three containers containing the shroud (to be referred to as sample 1) and two control samples (samples 2 and 3) were then handed to representatives of each of the three laboratories together with a sample of the third control (sample 4), which was in the form of threads. All these operations, except for the wrapping of the samples in foil and their placing in containers, were fully documented by video film and photography.

The laboratories were not told which container held the shroud sample. Because the distinctive three-to-one herringbone twill weave of the shroud could not be matched in the controls, however, it was possible for a laboratory to identify the shroud sample. If the samples had been unravelled or shredded rather than being given to the laboratories as whole pieces of cloth, then it would have been much more difficult, but not impossible, to distinguish the shroud sample from the controls. (With unravelled or shredded samples, pretreatment cleaning would have been more difficult and wasteful.) Because the shroud had been exposed to a wide range of potential sources of contamination and because of the uniqueness of the samples available, it was decided to abandon blind-test procedures in the interests of effective sample pretreatment. But the three laboratories undertook not to compare results until after they had been transmitted to the British Museum. Also, at two laboratories (Oxford and Zurich), after combustion to gas, the samples were recoded so that the staff making the measurements did not know the identity of the samples.

Controls

The three control samples, the approximate ages of which were made known to the laboratories, are listed below. Two were in the form of whole pieces of cloth (samples 2 and 3) and one was in the form of threads (sample 4).

Sample 2. Linen (sample QI.T/32) from a tomb excavated at Qasr Ibrîm in Nubia by Professor J. M. Plumley for the Egypt Exploration Society in 1964. On the basis of the Islamic embroidered pattern and Christian ink inscription, this linen could be dated to the eleventh to twelfth centuries AD.

Sample 3. Linen from the collection of the Department of Egyptian Antiquities at the British Museum, associated with an early second century AD mummy of Cleopatra from Thebes (EA6707). This linen was dated in the British Museum Research Laboratory using liquid scintillation counting, giving a radiocarbon age of 2,010 ± 80 yr BP (BM-2558). This corresponds to a calendar age, rounded to the nearest 5 years, of 110 cal BC–AD 75 cal at the 68 per cent confidence level[5] (where cal denotes calibrated radiocarbon dates).

Sample 4. Threads removed from the cope of St Louis d'Anjou which is held in a chapel in the Basilica of Saint-Maximin, Var, France. On the basis of the stylistic details and the historical evidence the cope could be dated at ~AD 1290–1310 (reign of King Phillipe IV).

Measurement procedures

Because it was not known to what degree dirt, smoke or other contaminants might affect the linen samples, all three laboratories subdivided the samples, and subjected the pieces to several different mechanical and chemical cleaning procedures.

All laboratories examined the textile samples microscopically to identify and remove any foreign material. The Oxford group

Table 1 Basic data (individual measurements)

	Sample 1		Sample 2		Sample 3		Sample 4		Pretreatment and replication codes
Arizona	**AA-3367**		**AA-3368**		**AA-3369**		**AA-3370**		
	A1.1b*	**591 ± 30**	**A2.1b**	**922 ± 48**	**A3.1b**	**1,838 ± 47**	**A4.1b**	**724 ± 42**	
	A1.2b	**690 ± 35**	**A2.2a**	**986 ± 56**	**A3.2a (1)**	**2,041 ± 43**	**A4.2a**	**778 ± 88**	**a, method a**
	A1.3a	**606 ± 41**	**A2.3a (1)**	**829 ± 50**	**A3.3a**	**1,960 ± 55**	**A4.3a (1)**	**764 ± 45**	**b, method b**
	A1.4a	**701 ± 33**	**A2.4a (2)**	**996 ± 38**	**A3.4a(2)**	**1,983 ± 37**	**A4.4a (2)**	**602 ± 38**	**( ), same subsample**
			A2.5b	**894 ± 37**	**A3.5b**	**2,137 ± 46**	**A4.5b**	**825 ± 44**	
δ^{13}C (‰)		**−25.0**		**−23.0**		**−23.6**		**−25.0**	
Oxford	2575		2574		2576		2589		
	O1.1u	795 ± 65	O2.1u	980 ± 55	O3.1u	1,955 ± 70	O4.2u	785 ± 50	u, unbleached
	O1.2b	730 ± 45	O2.1b	915 ± 55	O3.1b	1,975 ± 55	O4.2b (1)	710 ± 40	b, bleached
	O1.1b	745 ± 55	O2.2b†	925 ± 45	O3.2b	1,990 ± 50	O4.2b (2)	790 ± 45	( ), same pretreatment/ run combination
δ^{13}C‡ (‰)		−27.0		−27.0		−27.0		−27.0	
Zurich	**ETH-3883**		**ETH-3884**		**ETH-3885§**		**ETH-3882**		
	Z1.1u	**733 ± 61**	**Z2.1u**	**890 ± 59**	**Z3.1u**	**1,984 ± 50**	**Z4.1u**	**739 ± 63**	
	Z1.1w	**722 ± 56**	**Z2.1w**	**1,036 ± 63**	**Z3.2w**	**1,886 ± 48**	**Z4.1w**	**676 ± 60**	**u, ultrasonic only**
	Z1.1s	**635 ± 57**	**Z2.1s**	**923 ± 47**	**Z3.2s**	**1,954 ± 50**	**Z4.1s**	**760 ± 66**	**w, weak**
	Z1.2w	**639 ± 45**	**Z2.2w**	**980 ± 50**			**Z4.2w**	**646 ± 49**	**s, strong**
	Z1.2s	**679 ± 51**	**Z2.2s**	**904 ± 46**			**Z4.2s**	**660 ± 46**	
δ^{13}C‖ (‰)		**−25.1**		**−23.6**		**−22.0**		**−25.5**	

In years BP, corrected for δ^{13}C fractionation; errors at 1σ level; see text for pretreatment details.

* The identification code for each measurement shows, in order, the laboratory, sample, measurement run, pretreatment and any replication involved.

† One anomalous replicate (of 6) obtained for independent measurement O2.2b; if rejected it reduces date by 40 yr; final date quoted actually reduced by 20 yr.

‡ Measured for samples 1 and 3; assumed for samples 2 and 4.

§ The loose weave of sample Z3.1 led to its disintegration during strong and weak chemical treatments. Z3.2 was centrifuged to avoid the same loss of material.

‖ Average of separate determinations by AMS.

cleaned the samples using a vacuum pipette, followed by cleaning in petroleum ether (40 °C for 1 h) to remove lipids and candlewax, for example. Zurich precleaned the samples in an ultrasonic bath. After these initial cleaning procedures, each laboratory split the samples for further treatment.

The Arizona group split each sample into four subsamples. One pair of subsamples from each textile was treated with dilute HCl, dilute NaOH and again in acid, with rinsing in between (method a). The second pair of subsamples was treated with a commercial detergent (1.5% SDS), distilled water, 0.1% HCl and another detergent (1.5% triton X-100); they were then submitted to a Soxhlet extraction with ethanol for 60 min and washed with distilled water at 70 °C in an ultrasonic bath (method b).

The Oxford group divided the precleaned samples into three. Each subsample was treated with 1M HCl (80 °C for 2 h), 1M NaOH (80 °C for 2 h) and again in acid, with rinsing in between. Two of the three samples were then bleached in NaOCl (2.5% at pH-3 for 30 min).

The Zurich group first split each ultrasonically cleaned sample in half, with the treatment of the second set of samples being deferred until the radiocarbon measurements on the first set had been completed. The first set of samples was further subdivided into three portions. One-third received no further treatment, one-third was submitted to a weak treatment with 0.5% HCl (room temperature), 0.25% NaOH (room temperature) and again in acid, with rinsing in between. The final third was given a strong treatment, using the same procedure except that hot (80 °C) 5% HCl and 2.5% NaOH were used. After the first set of measurements revealed no evidence of contamination, the second set was split into two portions, to which the weak and strong chemical treatments were applied.

All of the groups combusted the cleaned textile subsample with copper oxide in sealed tubes, then converted the resulting CO_2 to graphite targets. Arizona and Oxford converted CO_2 to CO in the presence of zinc, followed by iron-catalysed reduction to graphite, as described in Slota *et al.*[6]. Zurich used cobalt-catalysed reduction in the presence of hydrogen, as described by Vogel *et al.*[7,8].

Each laboratory measured the graphite targets made from the textile samples, together with appropriate standards and blanks, as a group (a run). Each laboratory performed between three and five independent measurements for each textile sample which were carried out over a time period of about one month. The results of these independent measurements (Table 1) in each case represent the average of several replicate measurements made during each run (samples are measured sequentially, the sequence being repeated several times). The specific measurement procedures for each laboratory are given by Linick *et al.*[9] for Arizona, by Gillespie *et al.*[10] for Oxford and by Suter *et al.*[11] for Zurich. Arizona and Oxford measured $^{14}C/^{13}C$ ratios by AMS and determined the $^{13}C/^{12}C$ ratios using conventional mass spectrometry. Zurich determined both $^{14}C/^{12}C$ and $^{13}C/^{12}C$ quasi-simultaneously using AMS only.

The conventional radiocarbon ages were all calculated using the procedures suggested by Stuiver and Polach[12], with normalization to $\delta^{13}C = -25‰$, and were accordingly reported in yr BP (years before 1950). The errors, which are quoted in Table 1 at the 1σ level (σ is standard deviation), include the statistical (counting) error, the scatter of results for standards and blanks, and the uncertainty in the $\delta^{13}C$ determination (Arizona includes the $\delta^{13}C$ error at a later stage, when combining subsample results; Oxford errors below 40 yr are rounded up to 40).

Results

On completion of their measurements, the laboratories forwarded their results to the British Museum Research Laboratory for statistical analysis. The individual results as supplied by the laboratories are given in Table 1. Each date represents a unique combination of pretreatment and measurement run and applies to a separate subsample, except where indicated by the identification code. From these data it can be seen that, for each laboratory, there are no significant differences between the results obtained with the different cleaning procedures that each used.

Table 2 Summary of mean radiocarbon dates and assessment of interlaboratory scatter

Sample	1	2	3	4
Arizona	646 ± 31	927 ± 32	1,995 ± 46	722 ± 43
Oxford	750 ± 30	940 ± 30	1,980 ± 35	755 ± 30
Zurich	676 ± 24	941 ± 23	1,940 ± 30	685 ± 34
Unweighted mean*	691 ± 31	936 ± 5	1,972 ± 16	721 ± 20
Weighted mean†	689 ± 16	937 ± 16	1,964 ± 20	724 ± 20
χ^2 value (2 d.f.)	6.4	0.1	1.3	2.4
Significance‡ level (%)	5	90	50	30

Dates are in yr BP. d.f., degrees of freedom.
* Standard errors based on scatter.
† Standard errors based on combined quoted errors.
‡ The probability of obtaining, by chance, a scatter among the three dates as high as that observed, under the assumption that the quoted errors reflect all sources of random variation.

The mean radiocarbon dates and associated uncertainties for the four samples, as supplied by each of the three laboratories, are listed in Table 2 and shown in Fig. 1. Also included in Table 2 are the overall unweighted and weighted means, the weights being proportional to the inverse squared errors as quoted by the laboratories. The underlying principle of the statistical analysis has been to assume that, unless there is strong evidence otherwise, the quoted errors fully reflect all sources of error and that weighted means are therefore appropriate. An initial inspection of Table 2 shows that the agreement among the three laboratories for samples 2, 3 and 4 is exceptionally good. The spread of the measurements for sample 1 is somewhat greater than would be expected from the errors quoted.

More quantitatively, to establish whether the scatter among the three laboratory means was consistent with their quoted errors, a χ^2 test was applied to the dates for each sample, in accordance with the recommended procedure of Ward and Wilson[13]. The results of this test, given in Table 2, show that it is unlikely that the errors quoted by the laboratories for sample 1 fully reflect the overall scatter. The errors might still reflect the uncertainties in the three dates relative to one another, but in the absence of direct evidence on this, it was decided to give the three dates for sample 1 equal weight in determining the final mean, and to estimate the uncertainty in that mean from the scatter of results.

As shown in Table 2, the unweighted mean of the radiocarbon age of sample 1 and its uncertainty are 691 ± 31 yr BP. The confidence limits for sample 1 were obtained by multiplying the uncertainty by t_d, the value of a Student's t distribution with d degrees of freedom at the appropriate probability level. The value of d, which lies between the inter- and intra-laboratory degrees of freedom—that is, between 2 and 9—was estimated at 5 on the basis of an analysis of variance on the 12 individual measurements supplied by the laboratories[14]. Individual measurements from a particular laboratory were weighted according to their inverse squared errors, but the contributions from different laboratories were weighted equally, thus ensuring consistency with Table 2. Thus for sample 1, where the error has been estimated from the scatter, ~68% and 95% confidence limits for the true radiocarbon date were found from the 1.1σ and 2.6σ errors about the unweighted mean respectively, the multiplying factors being obtained from standard tables of the t_5 distribution. However, for samples 2, 3 and 4, the limits were obtained in the usual way from 1σ and 2σ quoted errors about the weighted means, assuming normality.

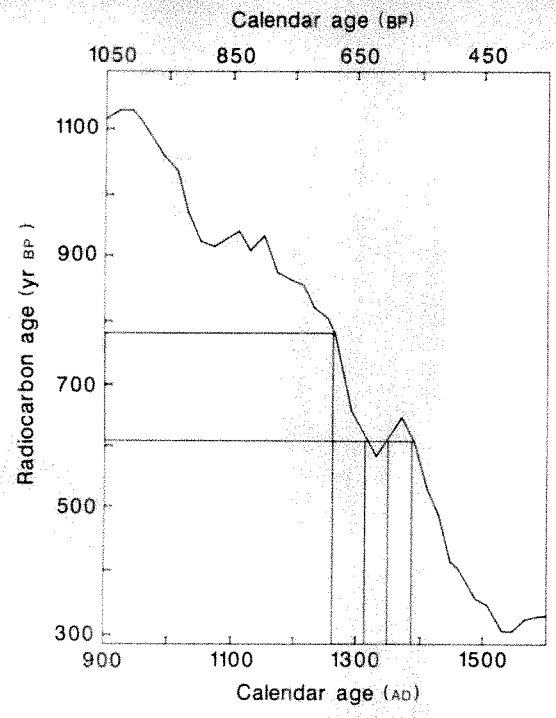


Fig. 2 Calibration of the overall mean radiocarbon date for sample 1 (the Shroud of Turin) using the 'intercept' method. (See also Table 3.) Calibration is necessary because of natural variations in atmospheric ^{14}C. The calibration curve for the relevant period is that of Stuiver and Pearson[5], a portion of which is illustrated. The uncertainty in the calibration curve has been combined with the error in the mean radiocarbon date, giving the 95% confidence limits on the radiocarbon scale; the error envelope on the curve has therefore been omitted from the diagram. The stippled areas show how the 95% confidence limits are transformed from the radiocarbon to the calendar scale.

Table 3 Calibrated date ranges at the 68% and 95% confidence levels

Sample	Mean date (yr BP)		Calendar date ranges
1*	691 ± 31	68%	AD 1273–1288
		95%	AD 1262–1312, 1353–1384 cal
2†	937 ± 16	68%	AD 1032–1048, 1089–1119, 1142–1154 cal
		95%	AD 1026–1160 cal
3†	1,964 ± 20‡	68%	AD 11–64 cal
		95%	9 cal BC–AD 78 cal
4†	724 ± 20	68%	AD 1268–1278 cal
		95%	AD 1263–1283 cal

* Confidence limits on the radiocarbon scale found from the standard error on the unweighted mean, assuming a t_5 distribution (multiplying factors 1.1 and 2.6 for 68% and 95% limits respectively). Standard error estimated from scatter.

† Confidence limits on the radiocarbon scale found from the standard error on the weighted mean, assuming a normal distribution (multiplying factors 1 and 2 for 68% and 95% limits respectively). Standard error computed from quoted errors.

‡ Date by conventional radiocarbon dating at the British Museum: 2010 ± 80 yr BP (BM-2558).

The calendar-age ranges which correspond to the radiocarbon confidence limits are shown in Table 3. These were determined from the high-precision curve of Stuiver and Pearson[5] based on dendrochronological dating. Method A (the intercept method) in revision 2.0 of the University of Washington Quaternary Isotope Laboratory Radiocarbon Calibration Program[15] was used. In this method, the error in the calibration curve is first incorporated into the radiocarbon error, thus widening the limits on the radiocarbon scale slightly; calendar ages are then found that correspond to these limits, without transforming the complete probability distribution of radiocarbon dates. No additional uncertainty has been added to take account of the short growth period of textile samples. There is little published guidance on this, although it has been suggested that 15 years should be added in quadrature to the overall uncertainty in the radiocarbon date for samples of growth period less than one year, such as linen. In general, such additional uncertainty would widen the 95% calendar limits by ~2–4 years at either end, except for sample 3 where the 9 cal BC limit would be changed to 34 cal BC.

The 95% limits for the shroud are also illustrated in Fig. 2, where it is apparent that the calibration of the radiocarbon date for sample 1 gives a double range. The correct transformation of probability distributions from the radiocarbon to the calendar scale is still subject to debate, there being two different methods of dealing with multiple intercepts. However, both methods agree that the major probability peak lies in the earlier of the two ranges, in the 68% range at the end of the thirteenth century. Sample 4 has a very narrow calendar range: this is due to the steep slope in the calibration curve at this point, and is an occasional instance of calibration reducing rather than increasing a confidence range. Sample 3 compares well with the date obtained by conventional radiocarbon dating; there is no evidence for a difference between the two results. The dates for samples 2 and 4 agree with the historical evidence, which places them in the eleventh to twelfth centuries and late thirteenth/early fourteenth centuries AD respectively.

The results, together with the statistical assessment of the data prepared in the British Museum, were forwarded to Professor Bray of the Istituto di Metrologia 'G. Colonetti', Turin, for his comments. He confirmed that the results of the three laboratories were mutually compatible, and that, on the evidence submitted, none of the mean results was questionable.

Conclusions

The results of radiocarbon measurements at Arizona, Oxford and Zurich yield a calibrated calendar age range with at least 95% confidence for the linen of the Shroud of Turin of AD 1260–1390 (rounded down/up to nearest 10 yr). These results therefore provide conclusive evidence that the linen of the Shroud of Turin is mediaeval.

The results of radiocarbon measurements from the three laboratories on four textile samples, a total of twelve data sets, show that none of the measurements differs from its appropriate mean value by more than two standard deviations. The results for the three control samples agree well with previous radiocarbon measurements and/or historical dates.

We thank Cardinal Anastasio Ballestrero for allowing us access to the shroud, Professor L. Gonella for his help and support throughout the project and Professor A. Bray for commenting on our statistical assessment of the data. We also thank Miss E. Crowfoot, T. G. H. James, Dr J. Evin, M. Prevost-Macillacy, G. Vial, the Mayor of Saint-Maximin and the Egypt Exploration Society for assistance in obtaining the three known-age control samples. Oxford thank P. H. South (Precision Processes (Textiles) Ltd, Derby) for examining and identifying the cotton found on the shroud sample; R. L. Otlet (Isotopes Measurement Laboratory, AERE, Harwell) for stable isotope ratio measurements on two samples; J. Henderson and the Department of Geology, Oxford Polytechnic for undertaking scanning electron microscopy, and SERC for the Special Research Grant which provided the primary support for the Radiocarbon Accelerator Unit. Zurich thank the Paul Scherrer Institut (PSI, CH-5234 Villigen) for technical and financial support. The AMS Programme at Arizona is partially supported by a grant from the NSF.

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Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1

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The crystal structure of the protease of the human immunodeficiency virus type 1 (HIV-1), which releases structural proteins and enzymes from viral polyprotein products, has been determined to 3 Å resolution. Large regions of the protease dimer, including the active site, have structural homology to the family of microbial aspartyl proteases. The structure suggests a mechanism for the autoproteolytic release of protease and a role in the control of virus maturation.

THE HIV-1 retrovirus has been shown to be the causative agent in AIDS[1,2]. In the course of viral replication, many retroviral structural proteins and enzymes are initially translated as polyproteins which undergo enzymatic cleavage to generate the functional proteins found in mature virions[3]. Genetic and biochemical studies have demonstrated that a virally encoded protease is responsible for the release of structural proteins from the *gag* gene product, and for the release of protease (implying autoproteolysis), reverse transcriptase and integrase from the gag-pol fusion protein[4-8]. Analysis of the sequences of retroviral proteases led to the suggestion that these enzymes were members of the aspartyl protease family, on the basis of the observed conservation of a characteristic Asp-Thr-Gly active-site sequence[9]. HIV-1 protease was found to be inhibited *in vitro* by pepstatin[10-12], a general inhibitor of aspartyl proteases, whose action operationally defines members of this class of enzymes[13]. Furthermore, single amino-acid substitutions of the HIV-1 protease sequence in which the highly conserved Asp 25 was converted to Asn[12,14], Thr[10] or Ala[15] resulted in the elimination of protease activity. Upon transfection into mammalian cells, a provirus containing one of these mutations (Asn 25) was shown to generate non-infectious virions containing unprocessed polyprotein[14]. Consequently, we have focused on HIV-1 protease as a therapeutic target, and have now determined the structure of this enzyme to accelerate the rational design of protease inhibitors as potential agents in the control of AIDS.

HIV-1 protease, with only 99 amino-acid residues[16-19], is the smallest of the retroviral proteases, and is much smaller than the microbial and mammalian aspartyl proteases, each of which contains approximately 325 residues. These pepsin-like enzymes contain homologous N- and C-terminal domains that may have evolved from a primordial gene duplication and fusion event[20,21]. The domains, which are related by an approximate twofold axis of symmetry, each contribute one Asp-Thr-Gly triad to the pseudo-symmetric active site. We show here that in HIV-1 protease, a strict crystallographic twofold relationship exists, in which each monomer contributes one Asp-Thr-Gly sequence to a truly symmetric active site. The structure of this active site, including the interaction of the catalytic aspartic acid residues across the twofold axis, closely resembles the active site of the pepsin-like aspartyl proteases.

Structure of HIV-1 protease

The isolation and crystallization of the HIV-1 protease have been described elsewhere[22]. Details of the structure determination are given in Table 1 legend. C_α coordinates will be deposited with the Brookhaven Protein Data Bank[23].

The tertiary structure of the HIV-1 protease monomer contains exclusively β-sheet, turn and extended polypeptide structural elements. Schematic descriptions of the protease structure are presented in Fig. 1. In the 3.0 Å multiple isomorphous replacement (MIR) electron density map from which this structure was derived, strong, continuous electron density is observed for all but the first five residues at the N terminal (Pro 1 to Leu 5). Chemical sequence analysis of washed crystals, however, confirmed the presence of the correct N-terminal sequence, beginning with Pro 1 (J. Rodkey, unpublished results). We conclude that the first five residues are present in our crystallized protein, but that they are disordered crystallographically, probably because of an inherent flexibility (see below). It should be noted that our current model leaves no regions of electron density in the MIR map unassigned.

In this crystal structure, the two monomer components of each HIV-1 protease dimer are related by a crystallographic twofold axis of symmetry. The overall shape of the dimer is very asymmetric; it can be approximated by an oblate ellipsoid, roughly 55 by 35 by 25 Å. To simplify the description of the dimer, the two monomers will arbitrarily be denoted A and B, even though they are crystallographically equivalent (Fig. 2). The dimer is stabilized by two sets of antiparallel interactions,

‡ Sadly, Dr Irving S. Sigal was killed in the destruction of Pan Am Flight 103 over Lockerbie, Scotland on 20 December 1988.

Table 1 HIV-1 protease heavy-atom refinement parameters

Derivative	Site	X	Y	Z	Occupancy*	B(Å²)
$(C_6H_5)Hg(C_2H_3O_2)$	1	0.046	0.026	0.052	80.0	54.0
	2	0.165	0.962	0.101	33.8	133.0
	3	0.108	0.005	0.056	8.7	1.7
$Pb(NO_3)_2$	1	0.859	0.849	−0.003	17.8	36.0

	Resolution (Å)								
	11.7	8.3	6.4	5.2	4.4	3.8	3.4	3.0	Total
Native									
Observations (no.)	50	104	171	264	369	474	490	177	2,099
Figure of merit	0.93	0.93	0.88	0.81	0.79	0.65	0.47	0.38	0.67
$(C_6H_5)Hg(C_2H_3O_2)$									
Phasing power†	4.74	5.31	5.57	3.18	2.65	2.35	2.08	1.78	3.03
R_{Cullis}†	0.28	0.24	0.30	0.38	0.51	0.52	0.75	0.69	0.43
$Pb(NO_3)_2$									
Phasing power†	2.10	2.65	3.03	2.02	1.43	1.48	0.00	0.00	1.86
R_{Cullis}†	0.45	0.41	0.35	0.51	0.62	0.65	0.00	0.00	0.51

HIV-1 protease from the NY5 isolate[36] (Pro 1 to Phe 99) was crystallized in space group $P4_12_12$; the crystals have cell constants $a = b = 50.29$ Å and $c = 106.80$ Å. The heavy-atom derivatives used in the structure determination were formed by soaking the crystals in 1 mM solutions of eithe phenyl mercury acetate or lead nitrate for up to 12 hours at 4°C. Before data collection, the crystals were washed and backsoaked for at least 1 hours in the crystallization buffer without dithiothreitol. Diffraction data were recorded beyond 3.0 Å resolution using a Nicolet X-ray area detecto and CuK_α radiation from a Rigaku RU-200 rotating anode X-ray source operating at 50 kV and 60 mA. Each data set was measured from a singl crystal as a series of 5 min, 0.25° oscillation frames. A total of 440 individual frames, the equivalent of a 110° sweep, was collected and processe using the XENGEN processing software[37]. The native data set had an average sixfold redundancy, with an overall R_{sym} of 0.063 to 3.0 Å resolution The derivative data sets were measured and processed to preserve the signal that results from anomalous dispersion effects. Each derivative dat set had an average threefold redundancy, with an overall R_{sym} of 0.062 for the phenyl mercury acetate derivative and 0.076 for the lead nitrat derivative. Unless otherwise stated, all subsequent calculations were performed using the computer program package PROTEIN[38]. Initial heavy-atom positions were determined from difference Patterson maps using a vector verification technique. $P4_12_12$ was shown to be the correct space grou from difference Fourier calculations using the two derivative data sets. MIR refinement of the heavy-atom sites gave the values shown above. A contoured mini-map of electron density was computed using native structure factor amplitudes and MIR phases, and a tracing of the polypeptid chain was derived from this map. A full set of coordinates was created from C_α guide points obtained from the mini-map and the known protei sequence[36]. These coordinates were adjusted into the MIR electron density using the graphics program FRODO[39]. Coordinates were refined usin the constrained/restrained refinement program CORELS[40] from a starting R-factor of 0.485 to an R-factor of 0.370 at 3.0 Å resolution in fou steps: the fitted coordinates were regularized, the model was refined as a rigid body, individual amino acids were refined as constrained objects and side-chain dihedral angles were refined. These coordinates are the basis for the discussion of the HIV-1 protease structure presented here. We have initiated refinement using the program XPLOR[41] followed by refinement using the program PROLSQ[42]. Currently the R-factor without the inclusion of solvent is 0.25 for a model with reasonable geometry. Further rebuilding of the model and refinement are in progress.

* Normalized to 80 electrons for the highest occupancy mercury site.

† Phasing power $= \{\Sigma F_H^2/\Sigma(F_{PH^{(obs)}} - F_{PH^{(calc)}})^2\}^{1/2}$ and $R_{Cullis} = \Sigma|F_{PH^{(obs)}} - F_{PH^{(calc)}}|/\Sigma|F_{PH^{(obs)}} - F_{P^{(obs)}}|$ (for centric reflections) where F_P, F_H and F_{PH} are the structure factor amplitudes of the parent molecule, heavy atom and derivative, respectively.

involving residues Asp A25, Thr A26 and Gly A27 with Gly B27, Thr B26 and Asp B25, and, similarly, Thr A91, Gln A92 and Ile A93 with Ile B93, Gln B92 and Thr B91. There is an additional kinked antiparallel interaction involving Gln A7, Arg A8 and Pro A9 and Asn B98, Leu B97 and Thr B96, although these residues seem to be too far apart for cross-chain hydrogen bonding at this stage of the analysis. Side chains also seem to contribute to dimer stabilization. In particular, the position and orientation of both Asp A25 (and B25) and Thr A26 (and B26) are consistent with the highly conserved hydrogen-bonding scheme observed in aspartyl proteases of known structure[24–26]. Hydrophobic packing of side chains (involving, for instance, Trp A6 with Phe B99 and Ile A50 with Ile B50) may also contribute to the stabilization of the quaternary structure. Although the HIV-1 protease sequence contains two cysteine residues, at positions 67 and 95, these residues do not form disulphide linkages in the structure, either within or between monomers.

Protease structures compared

The HIV-1 protease structure was systematically compared to the structures of the three aspartyl proteases, all of microbial origin, for which coordinates are available from the Protein Data Bank[23]: rhizopuspepsin[24], penicillopepsin[25] and endothiapepsin[26]. From this analysis we concluded firstly, that large regions of the HIV-1 protease, including residues in the protease active site, have structural analogues in the microbial aspartyl proteases, and secondly, that a four-stranded β-sheet in the HIV-1 protease dimer (two strands from each monomer) differs in orientation and connectivity from the corresponding six-stranded β-sheet (three strands from each domain) observed in the microbial aspartyl proteases.

Toh and coworkers[9] noted homology between portions of the amino-acid sequences of retroviral proteases and the active-site sequences of pepsin-like aspartyl proteases. HIV-1 protease has structural homology corresponding to this sequence homology. The C_α positions of the 15 residues which were predicted to be homologous by Toh and coworkers, including the catalytic Asp–Thr–Gly triad, can be aligned with a r.m.s. deviation of 1.32 Å or less (Table 2) with corresponding residues in rhizopus-pepsin, penicillopepsin and endothiapepsin.

This structural homology between HIV-1 protease and the microbial proteases extends beyond the active sites, as shown in Table 2. When a monomer of HIV-1 protease is compared to the N-terminal domains of the microbial proteases, 47 residues can be aligned within the same r.m.s. tolerance as found for residues in the active-site loops. The region of the N-terminal domain referred to as the flap because of its apparent flexibility was specifically excluded from this alignment, however. Residues Lys 43 to Gln 58 of HIV-1 protease constitute an analogous structure. The position of the flap with respect to the body of the HIV-1 protease structure resembles most closely the position of the equivalent residues in penicillopepsin. In that structure, residues in the flap participate in interactions with symmetry-related molecules in the crystallographic unit

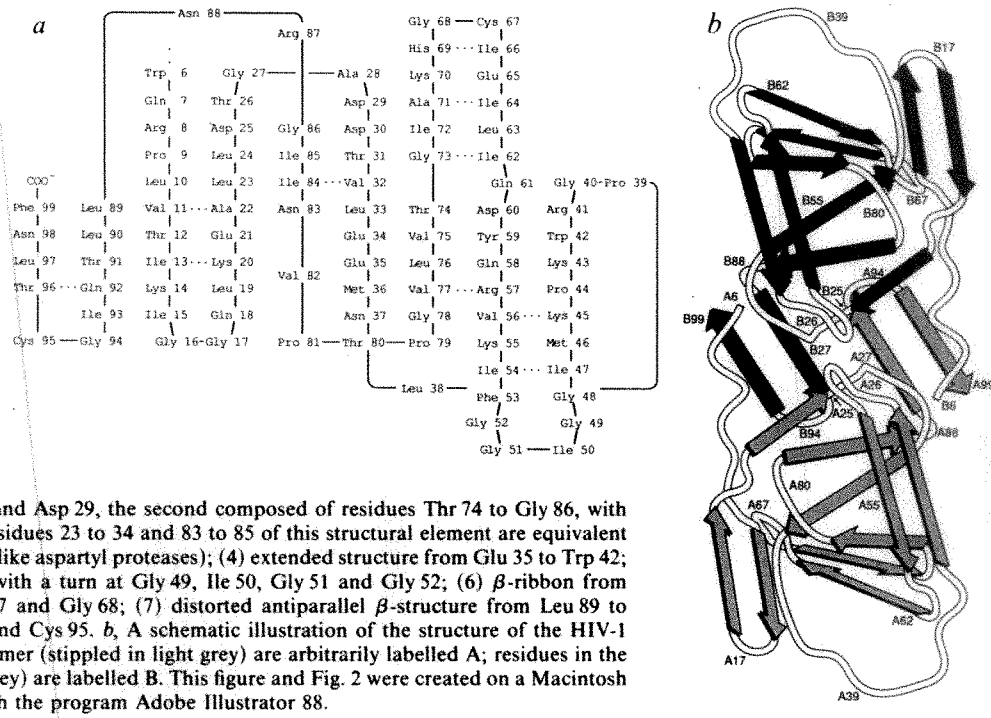


Fig. 1 *a*, A schematic illustration of the relationships between structural elements in the HIV-1 protease structure. Potential hydrogen bonds are indicated by dotted lines. Not indicated in this figure are potential hydrogen bonds between main-chain atoms of residues 65 and 67 and residues 12 and 14. As refinement proceeds, further hydrogen-bonding interactions will probably be revealed, in particular between residue Leu 23 and residues Asn 83 and Ile 85. The protein can be described as composed of seven structural elements: (1) extended structure from Trp 6 to Leu 10; (2) β-ribbon from Val 11 to Ala 22, with a turn at residues Gly 16 and Gly 17; (3) two interdigitating antiparallel structures, one composed of residues Leu 23 to Glu 34, with a turn at Gly 27, Ala 28 and Asp 29, the second composed of residues Thr 74 to Gly 86, with a turn at Pro 79, Thr 80 and Pro 81 (residues 23 to 34 and 83 to 85 of this structural element are equivalent to the Ψ-loop described for the pepsin-like aspartyl proteases); (4) extended structure from Glu 35 to Trp 42; (5) β-ribbon from Lys 43 to Gln 58, with a turn at Gly 49, Ile 50, Gly 51 and Gly 52; (6) β-ribbon from Ile 62 to Gly 73, with a turn at Cys 67 and Gly 68; (7) distorted antiparallel β-structure from Leu 89 to Phe 99, with a turn at Ile 93, Gly 94 and Cys 95. *b*, A schematic illustration of the structure of the HIV-1 protease dimer. Residues in one monomer (stippled in light grey) are arbitrarily labelled A; residues in the second monomer (stippled in darker grey) are labelled B. This figure and Fig. 2 were created on a Macintosh II with the program Adobe Illustrator 88.

cell[25]; this is also the case in the HIV-1 protease crystal structure. As shown in Fig. 3*b*, the position of the HIV-1 protease flap is more distant from the body of the structure than is the case in rhizopuspepsin. Flaps in the microbial proteases have been observed to adopt a variety of conformations and we anticipate that a similar distribution of conformations is available to HIV-1 protease.

Only 29 residues in an HIV-1 protease monomer can be aligned with residues in the C-terminal domains of microbial proteases with a tolerance of 1.45 Å or less. We do not include in this alignment the β-ribbon that contains residues 62–70. There is a corresponding structural element in C-terminal domains (residues 260–268 in rhizopuspepsin), but in HIV-1 protease this β-ribbon adopts a different orientation with respect to the body of the structure. The linkage between residues 74–77 and 83–85 (the crosspiece strand of the Ψ-loop) in HIV-1 protease contains five amino acids in a truncated square corner. This structural element resembles the corresponding feature in the C-terminal aspartyl protease domains (with six residues in a similar conformation) more than the corresponding feature in the N-terminal domains (where 13 or 14 residues adopt an extended conformation).

When the HIV-1 protease dimer is aligned with the intact microbial proteases, the r.m.s. deviation of the fit increases significantly (Table 2). This increased deviation reflects the fact that although the HIV-1 protease dimer is truly twofold sym-

Table 2 Comparison of the HIV-1 protease structure to the structures of three aspartyl proteases

	Active site r.m.s. (Å)	Active site residues	Domain r.m.s. (Å)	Domain residues					
HIV-1 protease monomer		**20–34**		**10–15**	**17–35**	**56–59**	**62–71**	**74–78**	**83–85**
Rhizopuspepsin N-terminal domain	1.32	30–44	1.29	18–23	27–45	85–88	90–99	103–107	121–123
Penicillopepsin N-terminal domain	1.20	28–42	1.27	18–23	25–43	83–86	88–97	101–105	120–122
Endothiapepsin N-terminal domain	1.29	27–41	1.27	15–20	24–42	82–85	87–96	100–104	119–121
HIV-1 protease monomer		**20–34**		**9–13**	**18–34**	**74–77**	**83–85**		
Rhizopuspepsin C-terminal domain	1.07	213–227	1.26	195–199	211–227	288–291	297–299		
'enicillopepsin C-terminal domain	1.16	208–222	1.43	191–195	206–222	286–289	296–298		
ndothiapepsin C-terminal domain	1.26	210–224	1.45	190–194	205–224	286–289	300–302		

V-1 protease dimer	r.m.s. (Å)	Residues A10–A15	A17–A35	A56–A59	A62–A71	A74–A78	A83–A85	B9–B13	B18–B34	B74–B77	B83–B85
act rhizopuspepsin	1.56	18–23	27–45	85–88	90–99	103–107	121–123	195–199	211–227	288–291	297–299
act penicillopepsin	1.60	18–23	25–43	83–86	88–97	101–105	120–122	191–195	206–222	286–289	296–298
act endothiapepsin	1.71	15–20	24–42	82–85	87–96	100–104	119–121	190–194	205–224	286–289	300–302

he C_α coordinates of the residues in the current model of the HIV-1 protease structure were aligned with the C_α coordinates of each aspartyl ease by a least-squares rigid-body procedure. In each comparison, the r.m.s. deviation of the aligned C_α positions is given as a measure of the ity of the fit. In the comparison with intact aspartyl proteases, the HIV-1 protease dimer was treated as a rigid body, as were the pepsin-like rtyl proteases. The indicated residues in each monomer (denoted arbitrarily as A and B) of the rigid dimer were simultaneously aligned with ues in the N- and C-terminal aspartyl protease domains.

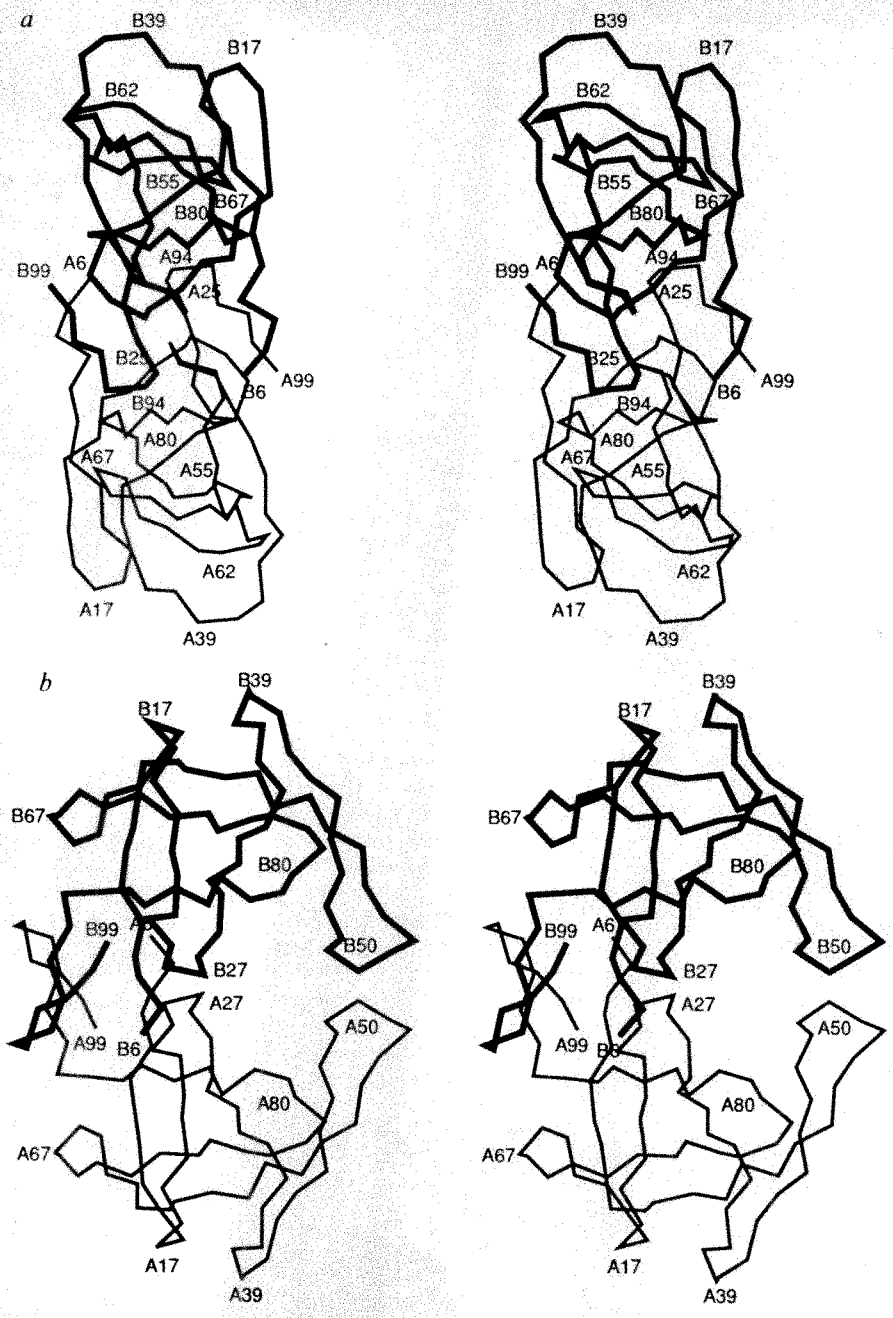


Fig. 2 A C_α representation of the structure of residues 6–99 of the HIV-1 protease dimer, with one monomer (arbitrarily labelled A) drawn in thin lines and the second monomer (arbitrarily labelled B) drawn in thick lines. *a*, The view is along the twofold symmetry axis of the protease dimer. *b*, The view represents a rotation of 90° about the vertical axis of the view of (*a*).

metric, the twofold axis that relates the N- and C-terminal domains of microbial aspartyl proteases is only approximate. (The relationship in penicillopepsin has been described[20] as a rotation of 174.6° and a translation of 1.0 Å.) As Fig. 3 illustrates, the most striking difference between the superimposed structures occurs in the orientation and connectivity of the β-sheet formed by residues at the N and C terminals (note that these regions are not included in the rigid body superposition).

The topological homology between an HIV-1 protease monomer and the N- and C- terminal domains of the microbial aspartyl proteases involves the same residues that are homologous between the two domains. There are 85 pairs of residues in the N- and C-terminal domains of rhizopuspepsin that can be aligned with an r.m.s. deviation for the C_α positions of 2.3 Å[24]. If we exclude residues in the six-stranded sheet from this list, 57 pairs of residues remain. All 47 of the residues used in the N-terminal alignment of Table 2 are in this set of domain-equivalent residues, as are all 29 of the residues used in the C-terminal alignment.

We have also compared the structure of the HIV-1 protease reported here to the model for the structure proposed by Pearl and Taylor[27]. To the extent that the X-ray structure is simila to the microbial aspartyl protease structures, from which thei model was derived, it would seem that their predictions ar reasonable. There are significant differences between the stru ture and their model, however. These differences include th topology of the N- and C-terminal regions and the predicti of helical structure near the C terminal (compare their Fig. with our Fig. 1*a*).

Release from the *gag-pol* product

The HIV-1 protease monomer is synthesized as part of a *gag* fusion product which results from an infrequent ribosomal fra shift from the *gag* to the *pol* reading frames[28]. Evidence sugg that the protease can autocatalytically release itself from gag-pol polyprotein[7,16,29]. The structure of the HIV-1 prot dimer presented here suggests a mechanism by w autoproteolysis at its N-terminal connections to the polypr may occur. From the observed position of residue Trp 6 i structure and the implied flexibility of the five N-ter residues (see above), it seems that the scissile Phe–Pro b could be positioned in the protease active site for cleavage

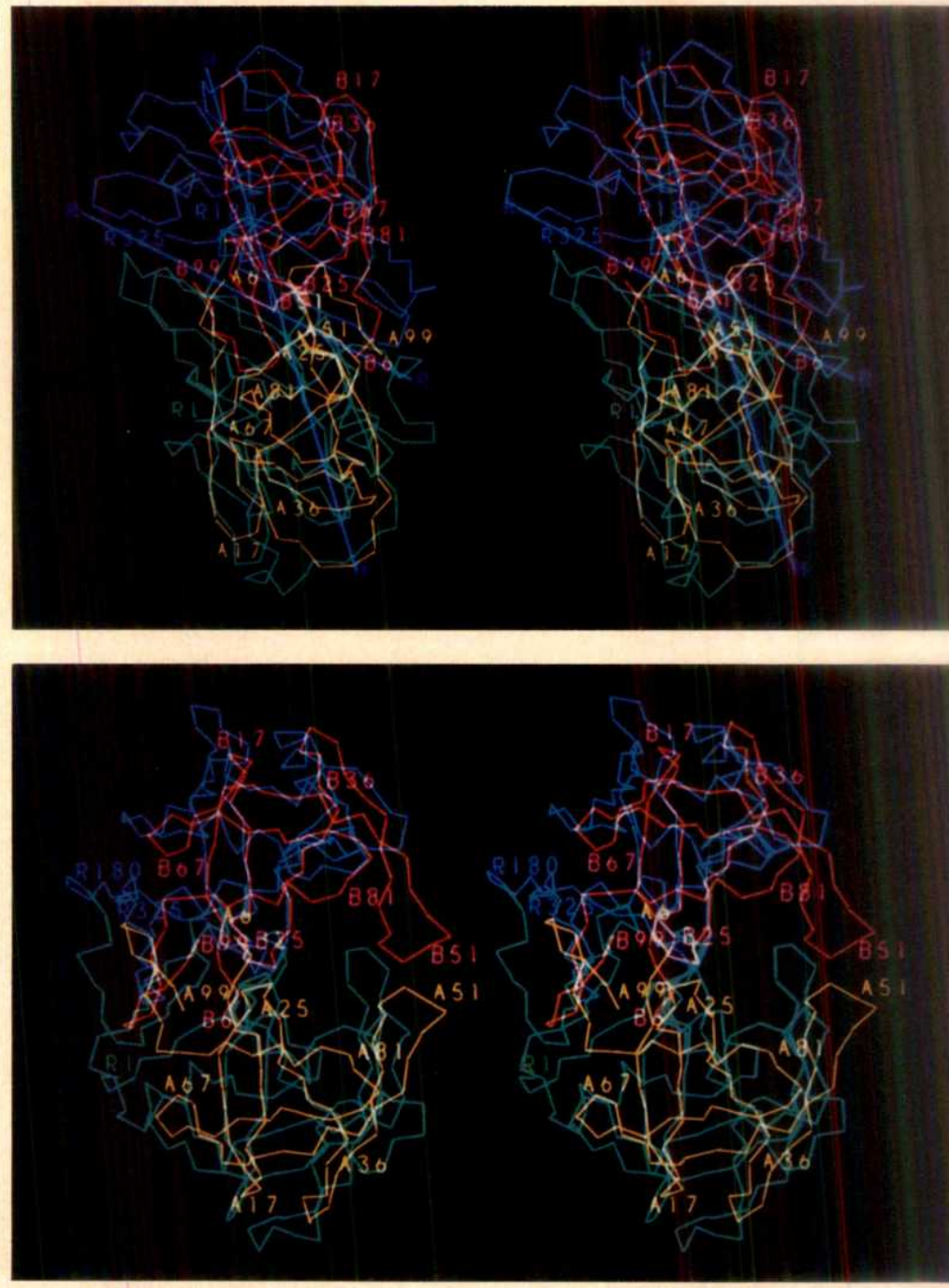


Fig. 3 A comparison of the structure of the HIV-1 protease dimer with rhizopuspepsin. 47 residues in monomer A of HIV-1 protease (gold) have been aligned with the corresponding residues in the N-terminal domain of rhizopuspepsin (green); 29 residues in monomer B of the protease (red) have been aligned with residues in the C-terminal domain (blue). The views in (*a*) and (*b*) are as in Fig. 2. The lines in Fig. 3*a* emphasize the approximate 50° difference in orientation of the multistranded β-sheets in the two structures (R indicates rhizopuspepsin, H indicates HIV-1 protease). The connectivity of the sheets is also different. The N-terminal domain of rhizopuspepsin contributes strands to the half of the sheet that lies to the lower left of the approximate dimer axis when viewed down that axis; the C-terminal domain contributes strands that lie to the upper right. In contrast, monomer A of the HIV-1 protease dimer, which has been aligned with the N-terminal domain of rhizopuspepsin, contributes strands to the half of the sheet that lies to the right of the dimer axis; monomer B, aligned with the C-terminal domain, contributes strands that lie to the left.

pol-encoded residues between the frameshift site and the protease have no known structural or catalytic function. They may, in fact, serve as a flexible linker to facilitate the N-terminal cleavage process. All of this implies that the N-terminal clips needed to release the protease, could be accomplished intramolecularly.

Analogous C-terminal cleavages are more difficult to envisage because this sort of cleavage would require the unravelling of a substantial portion of the structure to position in the active site the bond connecting the C-terminal Phe 99 of HIV-1 protease to the directly adjacent N-terminal Pro 1 residue of the much larger reverse transcriptase. We suggest that the cleavage at the C terminal can be carried out intermolecularly by a second HIV-1 protease dimer.

Control of HIV-1 assembly

The process of viral assembly takes place at the plasma membrane of the host cell. Both the *gag* and *gag-pol* products migrate there and become anchored to the cytoplasmic side by a covalent, N-terminal-linked myristic acid[30,31]. Electron micrographs of the virus assembly process suggest that the polyproteins aggregate, the membrane bulges outward and nascent viral particles bud from the plasma membrane[32]. The generation of mature viral enzymes and structural proteins from these polyproteins does not seem to occur until after the virion has budded[14,32]. The conversion of the immature virion to the morphologically distinct, infectious entity requires the participation of HIV-1 protease[14].

By analogy with the pepsin-like aspartyl proteases, only a dimer of HIV-1 protease would be catalytically competent. The requirement that dimers form before proteolysis can occur indicates a possible role for HIV-1 protease in the control of virus maturation. If an active protease dimer were to form before the nascent virus had budded from the host cell, autoproteolysis at the N terminal could occur. The symmetric nature of the protease active site allows this clip to occur for each of the two anchoring

gag-pol products, effectively solubilizing the protein, and permitting the protease (and the C-terminal linked enzymes) to diffuse away from the plane of the membrane. This diffusion would prevent premature initiation of virus maturation. Once the nascent virus particles have budded from the cell membrane, however, autoproteolytically liberated protease could diffuse only within the isolated environment of the virus particle. A cascade of proteolytic events, liberating gag and pol structural proteins and enzymes (including additional copies of the protease itself) would be initiated. Such a proteolytic cascade in the confined space of the viral envelope would generate a burst of mature viral proteins and allow the rapid self-assembly necessary to achieve full infectivity. If this mechanism is correct, it implies that retroviral proteases, including HIV-1 protease, may be more than 'fossil' examples of an ancestral dimeric aspartyl protease[27].

Conclusions

Self-assembly of two identical monomers into a symmetric structure represents an elegant method for creating an active enzyme while encoding a minimal amount of genetic information. The proposed mechanisms of action of the aspartyl proteases[33-35] require the exact symmetry observed in the HIV-1 protease dimer to be broken at some point during the binding and catalysis of intrinsically asymmetric peptidyl substrates. Because of the symmetric nature of the active site, however, the initial orientation of substrate can be either right-to-left or left-to-right in the view of Fig. 2*a*.

HIV-1 protease is the first of the HIV-1 proteins to have its structure determined. The close resemblance between its active sites and those of the pepsin-like aspartyl proteases, where many inhibitor complexes have been investigated crystallographically suggests that it may be possible to carry over the insights derived from these studies to the design of specific HIV-1 protease inhibitors, for use as safe and effective agents in the control of AIDS.

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Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria

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A nuclear encoded mitochondrial heat-shock protein hsp60 is required for the assembly into oligomeric complexes of proteins imported into the mitochondrial matrix. hsp60 is a member of the 'chaperonin' class of protein factors, which include the Escherichia coli *groEL protein and the Rubisco subunit-binding protein of chloroplasts.*

MOST mitochondrial proteins are encoded by nuclear genes, synthesized in the cytosol as precursor proteins and subsequently imported into the organelles (for reviews see refs 1-3). Newly synthesized precursors have to assume an 'unfolded' conformation to be translocated across the mitochondrial membranes[4-6]. Cytosolic heat-shock proteins of the hsp70 family plus an additional activity which might be an ATP-dependent 'unfoldase' may have a role in maintaining this translocation-competen conformation[7-12]. Transport across the mitochondrial membranes occurs at translocation contact sites between outer an inner membranes[13,14]. Proteolytic cleavage of presequences performed during or after translocation by the mitochondri

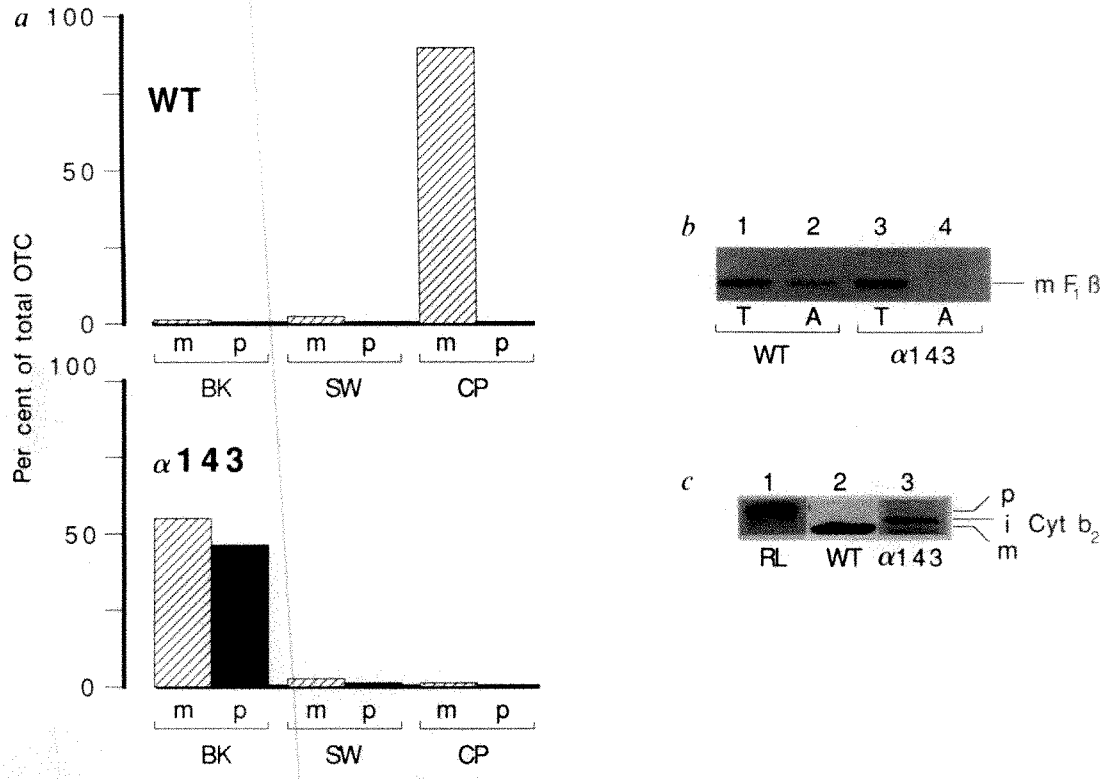


Fig. 1 Analysis of mitochondrial proteins in α143 cells. *a*, Defective assembly of OTC. Amounts of OTC contained in breakthrough (BK), salt wash (SW), and carbamyl phosphate elution (CP) of PALO columns are expressed as percentages of total OTC in cell extracts. p, Precursor; m, mature-sized OTC. *b*, Defective assembly of $F_1\beta$-ATPase. Lanes 1 and 3, immunoprecipitation of $F_1\beta$-ATPase from total amount (T) of mitochondrial fractions equivalent to the amounts analysed by chloroform extraction; lanes 2 and 4, immunoprecipitation of aqueous phase (A) of chloroform extracts. *c*, Defective maturation of cytochrome b_2. Lane 1, precursor of cytochrome b_2 immunoprecipitated after synthesis in a reticulocyte lysate (RL) directed by mRNA transcribed from the cytochrome b_2 gene cloned into pGEM3 (refs 20, 41); lane 2, mature-sized cytochrome b_2 immunoprecipitated from [^{35}S]methionine-labelled wild-type cells (WT); lane 3, intermediate-sized cytochrome b_2 immunoprecipitated from α143 cells.

Methods. *a*, Yeast cells were grown at 23 °C in YPEG medium until $A_{600} = 1$. Five A_{600} units of cells were resuspended in 1 ml fresh YPEG medium. After 30 min at 37 °C, galactose was added and incubation carried on for 2 h. Spheroplasts were then prepared[18], extracted with 20 mM Hepes, *p*H 7.4/1% Lubrol, and analysed on PALO columns[30]. Eluted fractions were precipitated with trichloroacetic acid (TCA) and analysed by SDS gel electrophoresis[42] and immunoblotting using anti-OTC antiserum and ^{125}I-protein A. Fluorographs were quantified by densitometry[20]. *b*, For *in vivo* labelling cells were grown at 23 °C in minimal galactose medium to an OD_{600} of 0.5. Four A_{600} units of cells were resuspended in 2 ml fresh minimal galactose medium and shifted to 37 °C. After 2 h, 400 μCi [^{35}S]methionine was added and incubation carried on for an additional 30 min. The reaction was stopped by adding NaN_3 to 10 mM and 25 OD_{600} units of nonlabelled cells as carrier. Spheroplasts were prepared and a crude mitochondrial fraction was isolated[18,20]. 5×10^6 c.p.m. of wild-type and 10×10^6 c.p.m. of α143 mitochondrial fractions were adjusted to 100 μl with 0.6 M sorbitol buffer and extracted with 50 μl chloroform[31] by vortexing for 1 min. Approximately 7.5% of total counts in wild-type and 4.5% in α143 were recovered in the aqueous phase. The aqueous phases were boiled in SDS-containing buffer and diluted 20-fold with TNTE (20 mM Tris *p*H 7.8, 150 mM NaCl, 1% Triton X-100 and 5 mM EDTA). $F_1\beta$-ATPase was immunoprecipitated[18] and the precipitates were analysed by SDS gel electrophoresis and fluorography.

processing peptidase in the matrix[15-18]. Some precursors of intermembrane-space proteins are re-translocated from the matrix back across the inner membrane by a mechanism similar to bacterial protein export[19,20]. Finally, all imported proteins have to (re)fold and in most cases assemble into supramolecular complexes to become functionally active. Very little is known about these last stages of the import pathway.

The complex processes involved in folding and assembly of proteins imported into mitochondria might not occur spontaneously but rather might require one or more gene products. To detect such components, a bank of temperature-sensitive lethal yeast mutants was screened for a phenotype which results from deficiency in assembly of mitochondrial proteins. We describe here a nuclear mutation, *mif4*, in which subunits of mitochondrial enzymes such as subunit β of F_1-ATPase are completely translocated into the organelles, are processed to their mature-sized forms but fail to assemble into their respective supramolecular complexes. Proteins with complex presequences like cytochrome b_2 and the Rieske Fe/S protein of complex III are also affected. These proteins normally pass through the matrix on their way to the intermembrane space and they probably require interaction with protein factors during their assembly process. In the *mif4* mutant they accumulate as incompletely processed import intermediates.

The *Mif4* gene codes for a constitutively expressed stress protein, hsp60, which is structurally related to the α-component of the chloroplast Rubisco subunit-binding protein and the *E. coli* heat-shock protein groEL[21,22]. These two proteins have been named 'chaperonins'[21,23] because they were proposed to chaperone oligomeric protein assembly, such as bacteriophage head assembly in *E. coli*[24,25], and Rubisco assembly in chloroplasts[26,27]. Yeast hsp60 is localized in the mitochondrial matrix as a high molecular weight complex which has a distinct sedimentation behaviour on sucrose gradients. We suggest that interaction of imported proteins with this hsp60 scaffold is an essential step in conferring a conformation to imported precursor proteins which renders them competent for assembly with other subunits or for further intramitochondrial sorting.

Mutants

Mutants lacking enzymatic activity of imported mitochondrial proteins. Recently, we described a procedure for the selection of so-called *mif*-mutants (*mif*, for mitochondrial import functions), conditional yeast mutants affecting the mitochondrial

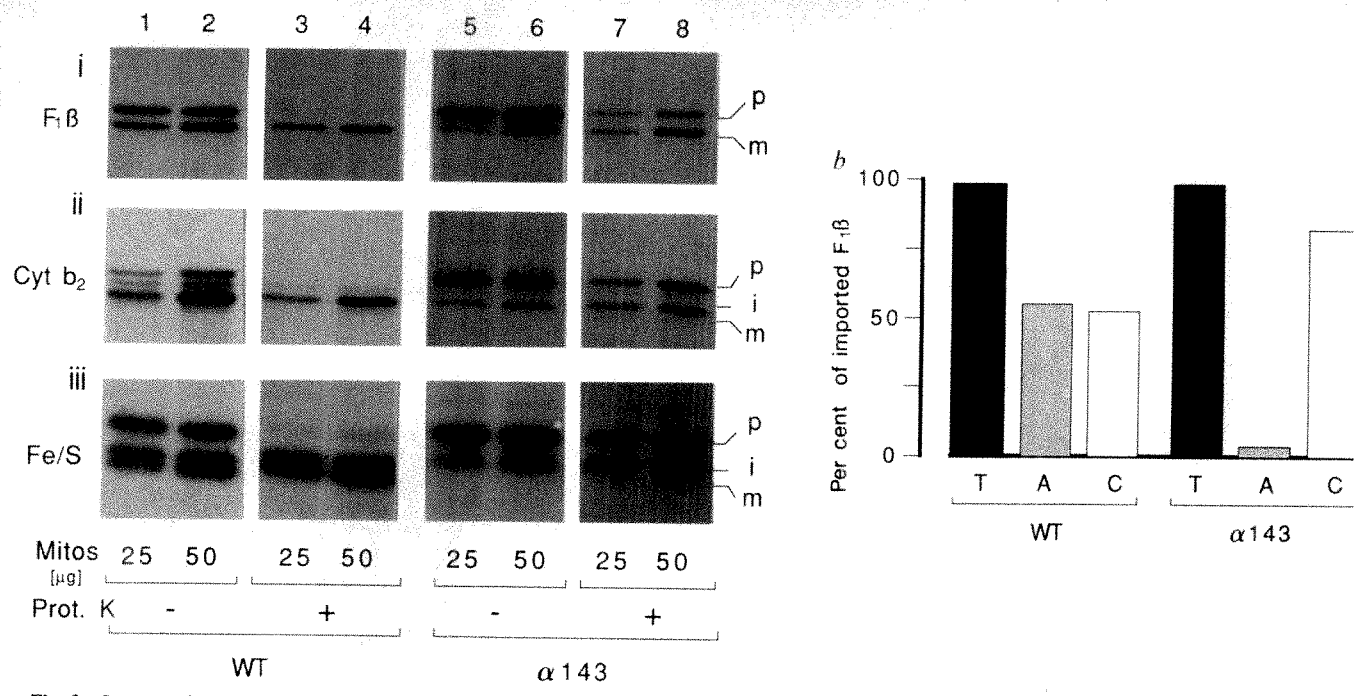


Fig. 2 Import of precursor proteins into isolated α143 mitochondria. *a*, Import of precursors of $F_1\beta$ (i), cytochrome b_2 (ii) and Rieske Fe/S protein (iii) into isolated mitochondria of wild-type (WT) and α143 mutant. Prot. K, proteinase K; p, precursor; i, intermediate-sized form; m, mature-sized form. *b*, Assembly of $F_1\beta$ imported *in vitro* into F_1-ATPase complex. Amounts of mature-sized $F_1\beta$ contained in chloroform (C) and aqueous phases (A) are given as percentages of total $F_1\beta$ contained in 50 μg of mitochondria (T) subjected to chloroform extraction. **Methods.** Wild-type and α143 yeast were grown overnight in YPEG at 23 °C. Then the temperature was shifted to 37 °C for 1 h. Spheroplasts were prepared and mitochondria were isolated[20]. Precursor proteins were synthesized in reticulocyte lysates in the presence of [^{35}S]methionine by coupled transcription/translation of the cDNAs ($F_1\beta$, Rieske Fe/S protein) or the genomic DNA (cytochrome b_2) cloned into pGEM3 using SP6 polymerase[20,41,43]. *a*, For import *in vitro* mitochondria were incubated for 30 min at 25 °C in reticulocyte lysates diluted 5-fold with BSA buffer (3% bovine serum albumin, 80 mM KCl, 10 mM MOPS (3-[*N*-morpholino]propanesulphonic acid) *p*H 7.2) containing 1 mM NADH and 2 mM ATP. The final concentration of mitochondria in the import reactions was 25 μg (lanes 1,3,5,7) and 50 μg (lanes 2,4,6,8) in a reaction volume of 100 μl. The reactions were placed on ice and one half of each sample was treated with proteinase K (Prot. K) (20 μg ml^{-1}, final concentration) for 30 min at 0 °C (lanes 3,4 and 7,8) (ref. 20). The other halves remained on ice for the same period. Finally, mitochondria were re-isolated, solubilized in SDS-containing buffer and analysed by SDS polyacrylamide gel electrophoresis and fluorography[20,42]. *b*, Mitochondria of wild-type and α143 cells (50 μg each) which had imported the radiolabelled precursor of $F_1\beta$ from a reticulocyte lysate were treated with protease as described above and subjected to chloroform extraction (see legend to Fig. 1*b*)[31]. Chloroform phases were dried under vacuum and aqueous phases precipitated with trichloroacetic acid (TCA) were analysed by SDS electrophoresis and fluorography. Fluorographs were quantified by densitometry.

protein import machinery[18]. Temperature-sensitive (ts) lethal mutants were derived by EMS-mutagenesis from a yeast strain (Ga1OTCRP/11) that is defective at the (cytosolic) ornithine transcarbamylase (OTC) locus (*arg3*) but contains the sequence coding for the human mitochondrial OTC (joined to an inducible yeast Ga11 promoter) integrated in its *URA3* locus. Upon expression in wild-type yeast cells, human OTC was imported into the mitochondrial matrix, processed and assembled to the enzymatically active trimer[28]. Mutants defective in expression of OTC activity at the non-permissive temperature were analysed. This led to the identification of mutants which accumulated at non-permissive temperature the precursor of human OTC but also the precursors of endogenous yeast mitochondrial proteins. This class of mutants included the complementation groups *mif1* and *mif2* which affect the processing enhancing protein (PEP) and the mitochondrial processing peptidase (MPP), respectively, components required for proteolytic processing of precursor proteins[16-18,29].

In a second class of ts-lethal mutants mature-sized OTC subunits accumulated. One mutant, α143, was further characterized. At non-permissive temperature the cells produced mature-sized OTC subunits and OTC precursor. But, no OTC activity was detectable. When examined at the permissive temperature, α143 cells behaved like wild-type. A single ts mutation which did not reside in the *OTC* gene itself was responsible for this phenotype.

Mutation affects assembly of several mitochondrial proteins. To test whether OTC subunits in the α143 cells had assembled into the homotrimeric enzyme an assay was carried out using the OTC substrate analogue δ-*N*-(phosphono-acetyl)-L-ornithine (PALO)[30] (Fig. 1*a*). Total extracts were prepared from cells grown for 2 h at 37 °C in galactose medium and were applied to columns containing PALO linked to epoxy-Sepharose. Extracts of wild-type cells contained only mature-sized OTC. Practically none of this was detected in the flow-through and wash fraction of the column, but OTC could be completely eluted with the OTC substrate carbamyl phosphate (Fig. 1*a*, upper panel). In contrast, with extracts of α143 cells both precursor and mature-sized OTC subunits were observed. They were quantitatively recovered in the flow-through (Fig. 1*a*, lower panel). The total contents of OTC polypeptides in wild-type and α143 cells were very similar. At non-permissive temperature mature-sized human OTC subunits were produced in α143 cells but failed to assemble into the homotrimeric, catalytically active, enzyme.

To find out whether the α143 mutation affects the assembly of an endogenous yeast mitochondrial protein we investigated the assembly of the β-subunit of F_1-ATPase ($F_1\beta$) into the F_1-ATPase complex (Fig. 1*b*). Our assay took advantage of the previous observation that upon treatment of a mitochondrial suspension with chloroform, assembled F_1-ATPase complex partitions into the aqueous phase, whereas non-assembled subunits of the ATPase partition into the organic phase[31]. Cultures of α143 and of wild-type cells were grown at 23 °C, then shifted to 37 °C and radio-labelled with [^{35}S]methionine. Mitochondria were isolated and were subjected to chloroform extraction

About 40–50% of total mature-sized $F_1\beta$ of wild-type mitochondria was recovered in the aqueous phase of the chloroform extraction, reflecting assembly of mature subunits into the ATPase complex (Fig. 1*b*, lane 2 versus 1). In contrast, virtually no mature $F_1\beta$ subunit of α143 mitochondria was detectable in the aqueous phase (Fig. 1*b*, lane 4 versus 3). We conclude that mature $F_1\beta$ subunits produced in the α143 mutant at the non-permissive temperature failed to assemble into the F_1-ATPase complex.

This observed deficiency in assembly of two matrix-located proteins in the α143 cells led us to ask whether the import pathway of proteins passing through the matrix compartment *en route* to the intermembrane space was also affected. One example of such a protein is cytochrome b_2. The precursor containing a bipartite targeting sequence is first completely translocated into the matrix and cleaved by the mitochondrial processing peptidase to an intermediate-sized form. This intermediate is directed back across the inner membrane by the second part of the presequence and cleaved to the mature-sized form at the outer surface of the inner membrane[20]. When extracts of α143 cells grown at non-permissive temperature were analysed, essentially only the intermediate-sized form was detected (Fig. 1*c*). These observations could be explained either by a defect in the translocation of the precursor into the matrix space or by a conformational alteration conferred to the precursor within the matrix compartment.

Block is after membrane translocation. To decide which step of the import pathway was affected by the α143 mutation the same proteins whose assembly had been examined in intact cells were synthesized as radiolabelled precursors in a reticulocyte lysate and added to isolated mitochondria. Upon incubation with wild-type mitochondria prepared from cells grown at 37 °C, precursor of $F_1\beta$ was converted to its mature-sized form which was protected against externally-added protease (Fig. 2*a*, panel *i*). When import into mitochondria from α143 cells grown for 1 h at 37 °C was examined, again the mature-sized subunit (and the precursor) was observed to be protected from digestion by added protease. This excluded a defect in translocation of precursor proteins. To determine the state of assembly of the imported mature $F_1\beta$ subunits, the organelles were re-isolated from the import reactions and subjected to chloroform extraction (Fig. 2*b*). With wild-type mitochondria, more than 50% of the imported mature-sized $F_1\beta$ partitioned into the aqueous phase. In contrast, after extraction of α143 mitochondria virtually no mature-sized $F_1\beta$ was detected in the aqueous phase, indicating that the imported subunit had failed to assemble.

Precursor of cytochrome b_2 was also imported by α143 mitochondria into a protease-protected position (Fig. 2*a*, panel ii). In contrast to intact cells, isolated mitochondria from α143 cells accumulated the precursor rather than the intermediate-sized form. This might reflect kinetic differences between import reactions *in vivo* and *in vitro*. The activity of the mitochondrial processing enzyme measured in detergent extracts of α143 mitochondria was not reduced compared to extracts prepared from wild-type organelles (data not shown).

In a similar way, the mutation affected assembly of the Rieske Fe/S protein of complex III. After translocation into the matrix, this protein, like cytochrome b_2, reaches a location at the outer surface of the inner membrane by re-translocation across the inner membrane. Fe/S precursor is proteolytically processed in two steps. In contrast to cytochrome b_2, however, the second processing event takes place in the matrix before re-transport to the intermembrane space. The second processing event which removes the carboxy-terminal eight residues of the presequence is probably dependent on a conformational change of the protein induced by the formation of the iron-sulphur cluster[19]. Isolated α143 mitochondria imported the precursor of Fe/S protein into a protease protected position but were unable to process it to the mature-sized form (Fig. 2*a*, panel iii). Precursor and intermediate-sized Fe/S protein accumulated. In view of the results

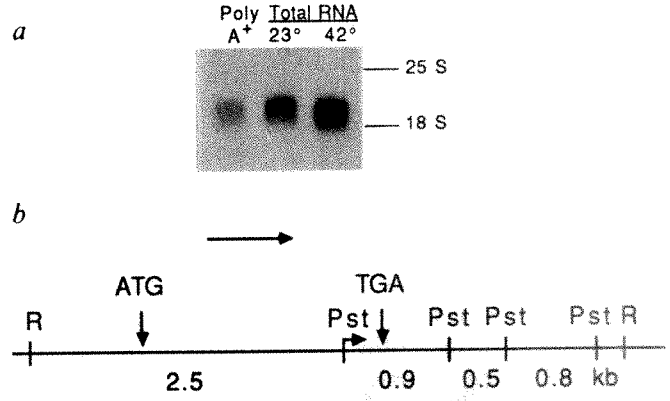


Fig. 3 Analysis of the rescuing plasmid p8. *a*, The insert of plasmid p8 recognizes a heat-inducible mRNA. 25 S and 18 S indicate migration positions of the respective yeast ribosomal RNAs. *b*, The restriction map of the p8 plasmid insert is identical to that of a λ-phage bearing the *Hsp60* gene. A 5 kilobase (kb) EcoRI fragment derived from plasmid p8 was analysed by *Pst*I digestion, and the fragment sizes compared with those of λ-Hsp60 (ref. 32). This latter clone had been isolated from a λgt11 yeast genomic library using an antiserum directed against the 58K mitochondrial heat-shock protein of *Tetrahymena thermophila*[33]. The fragment order is that indicated from sequence analysis of λ-Hsp60. A 0.9-kb *Pst*I fragment derived from p8 was sequenced in the region indicated by the horizontal arrow, and found to be identical to the one reported for the *Hsp60* gene[32]. The translational start and stop codons and direction of translation (long horizontal arrow) of *Hsp60* as determined from the clone are shown.
Methods. Wild-type cells were grown in YPEG medium at 23 °C to mid-log phase. Half of the culture was further incubated for 1 h at 23 °C and the other half at 42 °C. Total RNA was prepared[44]. Poly(A)$^+$ RNA was prepared from wild-type cells grown at 23 °C (ref. 45). RNA (10 μg of total, 2 μg of poly(A)$^+$) was electrophoresed in a 1% agarose gel, transferred to nitrocellulose[46], and hybridized with a nick-translated 0.9-kb *Pst*I fragment derived from the p8 insert (shown in Fig. 3*b*).

with import of the other mitochondrial proteins analysed, it is unlikely that the α143 mutation affects a mitochondrial component responsible for iron-sulphur cluster formation or the second proteolytic processing of Fe/S protein.

Altogether, the data suggest that α143 mitochondria are defective in a component which has a role in interacting with imported precursors, conferring conformational competence required for further steps of the import pathway. Such steps may include proteolytic processing, perhaps other covalent modifications (such as iron-sulphur cluster formation), assembly into supramolecular complexes, and further membrane translocation events.

Mutation is in the gene for hsp60. The α143 strain was mated at 23 °C with a wild-type strain of the opposite mating type and the diploids were shifted to 37 °C. They exhibited a wild-type phenotype indicating that the mutation in α143 is recessive. The α143 strain was next crossed with other recessive lethal mutant strains which are defective in mitochondrial import, belonging to the complementation groups of *mif1*, *mif2*, and *mif3* (ref. 18). Diploid cells from all three crosses grew normally at 37 °C, indicating that α143 belongs to a different complementation group, which we designated *mif4*.

To isolate a wild-type copy of the *Mif4* gene, the haploid α143 strain was transformed with a library containing yeast genomic DNA fragments inserted into a centromere-bearing plasmid. DNA was prepared from colony-purified transformants growing at 37 °C and used to transform *E. coli* to ampicillin-resistance[18]. Plasmids were isolated and found to share several restriction fragments of inserted yeast DNA.

To test whether the rescuing plasmids might encode a heat-shock protein, an insert from one plasmid (p8) was used to probe blots containing RNA prepared from wild-type yeast

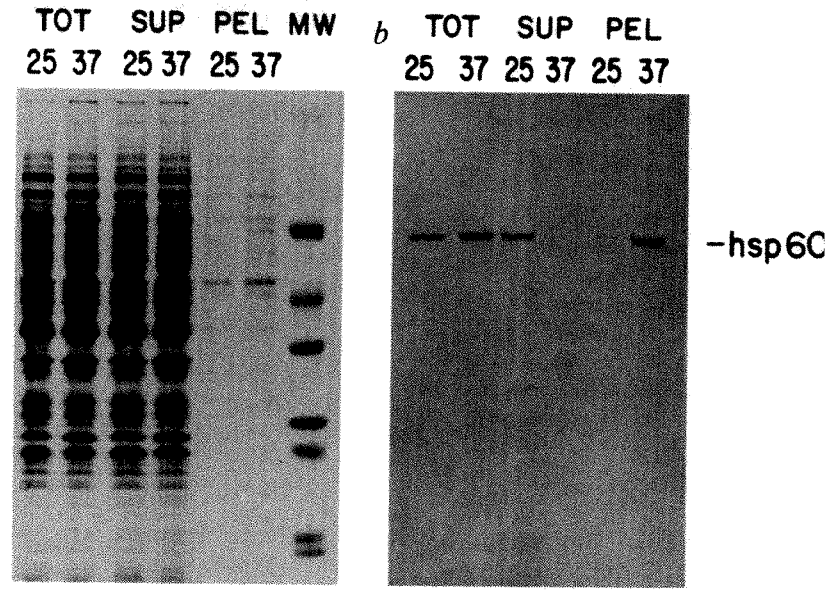


Fig. 4 Altered sedimentation of hsp60 protein in α143 cells. Fractionation by centrifugation of detergent extracts of α143 cells grown at 25 °C (25) or exposed to 37 °C for 2 h (37). *a*, Coomassie stained total proteins. *b*, Hsp60 as shown by immunoblotting using anti-hsp58 antiserum[33]. TOT, total extracts; SUP, supernatants; PEL; pellets.
Methods. An overnight culture of α143 cells grown at 25 °C was divided into halves. One half remained at 25 °C, whereas the other was transferred to 37 °C for 2 h. Then the cells were washed, broken by vortexing with glass beads and solubilized with 1% Triton X-100 and 0.5% deoxycholate[22,32]. After a clarifying centrifugation at 1,000*g* for 5 min, the extracts were centrifuged at 15,000*g* for 15 min. Equivalent amounts of total extracts, 15,000*g* supernatants and pellets were analysed on 12.5% SDS polyacrylamide gels.

maintained at 23 °C or exposed to 42 °C for 1 h. The insert identified a poly(A)$^+$ mRNA of ~1,800 nucleotides whose content in a preparation of total RNA was increased 2- to 3-fold by incubation of cells at 42 °C (Fig. 3*a*). It seemed possible that the *Mif4* gene encodes the heat shock protein (relative molecular mass, M_r, 60,000 (60K)) recently identified in mitochondria of yeast[22]. The restriction map of the insert p8 was compared with the restriction map of a λgt11 clone containing the *Hsp60* gene[32]. The maps of the two cloned yeast segments overlapped precisely (Fig. 3*b*). A region of p8 extending over 150 base pairs (bp) was subjected to DNA sequence analysis. The sequence was identical with that from the corresponding region in *Hsp60*, which lies within the protein-coding region[32] (data not shown).

To confirm that the *Hsp60* gene is the one affected by the *mif4* mutation a wild-type copy of *Hsp60* was integrated into the *Hsp60* locus in α143 cells and mating was carried out with a wild-type strain. Following sporulation, tetrad analysis revealed that all spores were viable at 37 °C. Thus, the *mif4* mutation responsible for the observed defect of assembly of mitochondrial proteins, is in the structural gene for hsp60.

Mutant contains an altered hsp60 complex. The *Hsp60* gene is essential for the growth of yeast and encodes a precursor (~61K) that is targeted to the mitochondrial matrix, probably by an amino-terminal cleavable targeting sequence[32]. The mature protein bears a distinct resemblance to the 58K product of the *groEL* gene of *E. coli*, a product required for bacteriophage head assembly and essential for cell viability[21,24]. Like groEL, hsp60 resides in a macromolecular complex[22]. Following extraction in its native state it sediments in sucrose gradients to a size position corresponding to a multimer of 12–16 identical subunits. In electron microscopic analysis of fractions containing the protein, two 7-fold symmetric 'doughnuts' are observed, placed one on top of the other[22].

If the macromolecular complex containing hsp60 is the functionally active form, then alteration of the hsp60 component by mutation might affect the integrity of the complex. Total extracts were prepared from α143 cells that had been either maintained at permissive temperature or shifted to 37 °C. Supernatants obtained by centrifugation at 15,000*g* were fractionated on sucrose gradients (data not shown). Hsp60 extracted from cells grown at 25 °C migrated as an approximately 20S particle. Analysis of extracts from α143 cells incubated at 37 °C for 30 min revealed a much smaller portion of the total hsp60 protein at this position of the gradient. Instead, most of the product was found in the pellet of the 15,000*g* centrifugation. When an extract from α143 cells which had been kept at 37 °C for 2 h was analysed, the total amount of hsp60 present increased about 2-fold but all of it was detected in the 15,000*g* pellet fraction (Fig. 4). In a control experiment with wild-type yeast, all of the hsp60 remained in the supernatant of the 15,000*g* centrifugation and migrated as 20S particles, irrespective of whether the cells were exposed to 37 °C. It is not known why hsp60 from α143 cells exposed to 37 °C sediments into a 15,000*g* pellet. The hsp60 complex may have become denatured and thus precipitated. Alternatively the complex might somehow have become associated with the mitochondrial membranes, although the presence of detergents during extraction makes this unlikely. Whatever the precise alteration, we conclude that the physical properties of the protein complex containing hsp60 are affected by the mutation present in the α143 strain.

Conclusions

Polypeptides entering the mitochondrial matrix space from the cytosol require a matrix protein, the *Mif4* gene product hsp60, for assembly and/or for further processing and sorting events. Consistent with this essential function, hsp60 is constitutively expressed. Its classification as a stress protein refers to the 2- to 3-fold induction observed in response to incubation of yeast cells at 42 °C. This response may reflect a cellular mechanism for maintaining functional conformation of mitochondrial proteins during and following heat stress.

The mechanism by which hsp60 affects the conformation of polypeptides entering the matrix space is undefined so far. The presence, however, of hsp60 in a macromolecular complex whose sedimentation properties are affected by mutation, could suggest that a 'machinery' is involved. Whether hsp60 is the only component of such a machinery is unknown. If the resemblance of hsp60 to the *E. coli* groEL protein[21] is any indicator, however, then other proteins are likely to play a role in addition, as in *E. coli* at least one other protein, groES, is required for assembly of phage heads[34,35]. Recently, the association of groEL with newly-synthesized, unfolded proteins has been demonstrated[36], suggesting that groEL might indeed have a function equivalent to its mitochondrial counterpart. Considering the double doughnut structure in which hsp60 apparently resides and the similar 14-subunit scaffold structure of groEL, we favour the idea that hsp60 may play mainly a structural role, acting as a 'workbench' on which folding and oligomerization is carried out by other proteins with catalytic activity. Putative catalytic components might reside either within the complex or within the soluble matrix. Included among such components could be another stress protein recently found to reside in the matrix space, belonging to the *Hsp70* gene family[37].

Based on the endosymbiont theory for the origin of mitochondria the utilization of hsp60 in the process of folding and assembly of imported proteins adds to the recently described evolutionarily conserved steps involved in the intramitochondrial sorting pathways of proteins of the intermembrane space[19,20]. Although the gene for hsp60 would have emigrated to the nuclear compartment at some point after the endosymbiotic event, the acquisition of a leader peptide permitted the gene product to remain in the mitochondria. An hsp60-related function may also be present in other cellular compartments of probable endosymbiotic origin. In chloroplasts, such a function is obviously present as indicated by structural similarity of the Rubisco subunit binding-protein α-subunit to both groEL and hsp60 and by its role in the assembly of the oligomeric Rubisco complex[21,26,27]. As defined by Ellis and colleagues[21,23] the groEL and Rubisco subunit-binding protein belong to one class of so-called 'molecular chaperones' termed 'chaperonins'. Our findings qualify the hsp60 protein of the mitochondrial matrix as a member of the 'chaperonin' class in a functional context. The involvement of protein factors, in particular heat-shock proteins, in folding and assembly of polypeptides has been suggested previously[10,38]. Some of these factors, such as cytosolic hsp70, may help in maintaining precursor proteins in a translocation-competent conformation. Other factors may control correct folding of newly synthesized polypeptides. The immunoglobulin heavy-chain binding protein (BiP), also known as GRP78 (refs 39, 40), might belong to this class of factors. The special function of the groEL/hsp60 family appears to be in actively catalysing the acquisition of the native conformation of oligomeric proteins. Our data strongly support the concept of protein-catalysed assembly of proteins entering the mitochondrial matrix space.

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LETTERS TO NATURE

Ultra-luminous OH maser emission from an IRAS galaxy

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Since the discovery[1] in 1982 of intense hydroxyl (OH) maser emission from the infrared-luminous galaxy Arp 220, twenty similar sources have been found in single-dish radio searches[2–7]. These so-called OH megamasers are associated with infrared-luminous galaxies with far-infrared colour temperatures of about 50 K. Here we report the detection of luminous OH maser emission in the galaxy IRAS20100–4156. This galaxy, at redshift $Z = 0.129$, displays similar optical properties to Arp 220, but exceeds it in OH luminosity by well over an order of magnitude, making it the most luminous OH maser known. The existence of such luminous megamasers increases the volume of space accessible for their study by a factor of about 50.

The generally accepted physical mechanism for OH emission in megamasers is the amplification of a centrally located continuum source by OH molecules in galactic molecular clouds[8]. The OH lines are most prominent at the main-line frequencies of 1,665 and 1,667 MHz and have velocity widths in excess of 100 km s^{-1}. Isotropic OH luminosities in the range 10^2–$10^3 L_\odot$ are typical, although a class of lower-luminosity sources (1–10 $L_\odot$) of somewhat smaller pumping efficiency is also known to exist (for example, NGC3690, NGC4418, IC5298).

Most searches for OH megamasers so far have been conducted using telescopes in the Northern Hemisphere. The only major search for sources in the Southern Hemisphere[9] was limited to galaxies with 60-μm-flux densities >10 Jy. We are now extending that survey to include IRAS galaxies with fluxes <10 Jy. The candidate objects have been selected by means of the distinctive far-infrared colours[10] of megamasers. Those which appear to be extragalactic, on the basis of identification from SERC sky survey films, are being searched for maser emission. Redshifts are being obtained with the Mt Stromlo 1.9-m reflector. A full description of this survey will be given on completion.

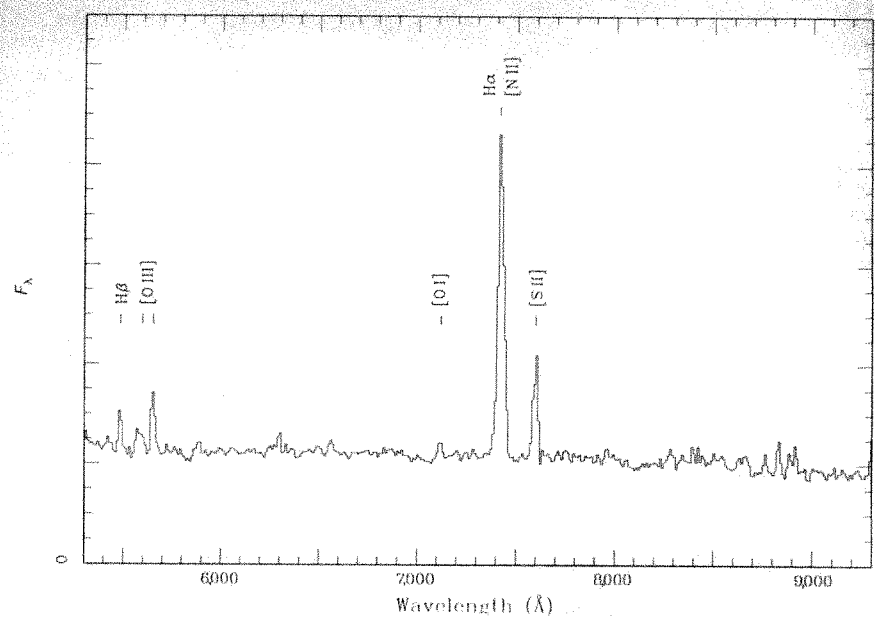


Fig. 1 Spectrum of IRAS20100-4156 taken with the Faint Object Red Spectrograph on the 3.9-m Anglo-Australian Telescope. Exposure time is 1,000 s and resolution is 20 Å. Vertical scale is flux per ångström in arbitrary units.

Low-dispersion optical spectra of IRAS20100-4156 were obtained during the period 5–9 September 1988 using a photon-counting array on the Mt Stromlo 1.9-m telescope. A resolution of 12 Å and a wavelength coverage of 3,600–8,000 Å were used. A further spectrum, shown in Fig. 1, was taken on 27 October 1988 during PATT service time on the 3.9-m Anglo-Australian Telescope (AAT) using the Faint Object Red Spectrograph to cover the wavelength range 5,300–9,300 Å. The emission lines detected were Hα/[N II], Hβ, [O I], [O II], [O III] and [S II]. The mean redshift of this object deduced from the AAT data, is 0.1291 ± 0.0002. From the line ratios, the object may be classified, using the diagrams of Veilleux and Osterbrock[11], as a low-ionization nuclear emission-line region (LINER). There is no indication of broad Seyfert-like lines.

Radio observations of IRAS20100-4156 were taken on 28 and 29 September 1988 using the Parkes 64-m telescope with the Australia Telescope prototype broad-band receiver. Typical system temperatures were 40 K. Spectra were obtained using the Parkes digital correlator. A bandwidth of 10 MHz was used, with 256 spectral channels in each of two orthogonal linear polarizations and in each of two frequency bands. An on-source integration time of 4,200 s was obtained for the redshifted OH 1,665- and 1,667-MHz main-lines, and of 3,000 s for the redshifted OH 1,720- and 1,612-MHz satellite line frequencies. Corresponding spectra are shown in Fig. 2. The velocity scale is heliocentric and is plotted in the optical convention ($v = c\Delta\lambda/\lambda_0$). The frequency resolution is 80 kHz, equivalent to a rest-frame velocity resolution of 16 km s^{-1} for the redshifted OH lines. Spectral interference, denoted by the gap in the spectrum in Fig. 2, was noted only for the 1,720-MHz observations.

Figure 2 reveals intense OH main-line emission with a peak flux density of ~200 mJy. The strongest emission peak, from the 1,667-MHz ($F = 2 \rightarrow 2$) transition, occurs at $38{,}697 \pm 3$ km s^{-1}; there is also a secondary peak at 38,648 km s^{-1}. A marginal detection of the 1,665-MHz ($F = 1 \rightarrow 1$) transition is also observed at 39,044 km s^{-1}, corresponding to a true velocity of 38,646 km s^{-1}, which is close to the velocity of the secondary blueshifted 1,667-MHz peak. No features are detected at the satellite line frequencies of 1,612 and 1,720 MHz. For the parabolic baseline fitted in Fig. 2, the 1,667:1,665-MHz peak-flux-density ratio is ≥ 8. Figure 2 also shows a pedestal of 1,667-MHz emission which extends over a velocity range of ~500 km s^{-1}. The total velocity range may, however, be considerably greater if, as we suspect, some of the parabolic baseline we have fitted is actually genuine line emission. Independent main-line spectra consistently appear to show extended emission over a total velocity range up to 1,500 km s^{-1}. However, because this velocity range is comparable with the total bandwidth of the observations, further observations are required to confirm it. OH emission over a broad velocity range (300–1,000 km s^{-1}) has been found in several other luminous megamasers[5,12,13]

Table 1 summarizes the properties of infrared and OH emission from IRAS20100-4156. A far-infrared (1–500 μm) luminosity of $3.0 \times 10^{12}\ L_\odot$ has been calculated[14], by ignoring the 12-μm contribution, for which only an upper limit is available, and by using a Hubble constant $H_0 = 75$ km s^{-1} Mpc^{-1}. In common with other strong OH emitters, IRAS20100-4156 is therefore extremely luminous in the far-infrared. On the basis of the SERC sky-survey films, IRAS20100-4156 seems to be compact, with an overall diameter of 10 arcsec. It shows a double structure, with components separated by 3 arcsec, corresponding to a projected linear separation of 8 kpc. The optical morphology is similar to that of Arp 220 and to other high luminosity infrared galaxies[15], and the unusual nature of this morphology suggests that, in common with most of these other galaxies, IRAS20100-4156 is a merger.

The OH luminosity of IRAS20100-4156 of $9.3 \times 10^3\ L_\odot$, obtained by subtracting the parabolic baseline in Fig. 2, considerably extends the luminosity range for megamasers. It is greater than the strongest megamasers known previously (Mrk

Table 1 Properties of IRAS20100-4156

Flux densities	100 μm	5.20 Jy
	60 μm	5.43 Jy
	25 μm	0.42 Jy
	12 μm	< 0.25 Jy
	OH 1,612 MHz	< 0.03 Jy
	OH 1,665 MHz*	0.02 Jy
	OH 1,667 MHz*	0.20 Jy
	OH 1,720 MHz	< 0.03 Jy
Redshifts	Optical	0.1291 ± 0.0002
	OH 1,667 MHz (peak)	0.12908 ± 0.00001
Luminosities†	40–120 μm‡	$2.0 \times 10^{12}\ L_\odot$
	1–500 μm§	$3.0 \times 10^{12}\ L_\odot$
	OH 1,667 MHz*	$9.3 \times 10^3\ L_\odot$

* Parabolic baseline fitted.
† $H_0 = 75$ km s^{-1} Mpc^{-1}, $q_0 = 1$.
‡ Formula from ref. 19.
§ Formula from ref. 14.

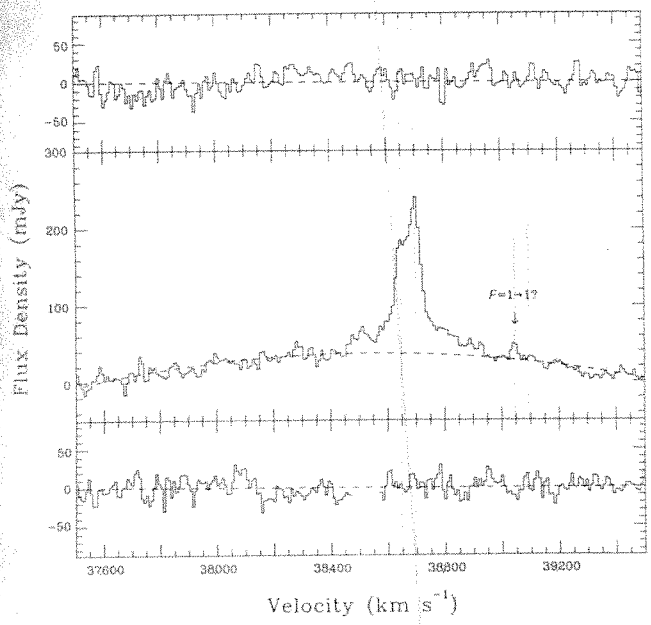


Fig. 2 Spectra of IRAS20100-4156 for the hyperfine transitions of OH at wavelength 18 cm, taken with the Parkes telescope. Velocities are in the optical convention ($c\Delta\lambda/\lambda_0$) with respect to a heliocentric rest frame. The dashed lines represent the instrumental baselines fitted to each of the three spectra. For the two satellite lines (top ($F=1\rightarrow2$) and bottom ($F=2\rightarrow1$)) these correspond to a constant flux density. For the main-lines (middle), a parabolic baseline is fitted. Some of the latter may, however, be genuine line emission (see text). The vertical dotted lines plotted at 38,648 and 38,697 km s^{-1} indicate the velocities of the two emission peaks in the $F=2\rightarrow2$ transition.

231, IRAS12112+0305 and IRAS17208-0014) by almost a factor of ten. The pumping efficiency given by the flux density ratio of OH emission to 60-μm emission, $f_{OH}/f_{60}=0.04$, is also higher than that of other megamasers. Such a high apparent efficiency provides a firm constraint for theoretical pumping models and, in particular, does not support the standard main-line pumping schemes[16,17]. For IRAS20100-4156 there is, however, no significant deviation from the quadratic relationship between OH and far-infrared luminosity which has been postulated[18] for megamasers.

The extreme luminosity of IRAS20100-4156 raises the prospect of detecting similar sources at much higher redshifts. At Parkes, sensitivity considerations limit the maximum attainable redshift to ~0.4 for such sources. This is also the limit at which it becomes difficult to select possible candidates from the IRAS Point Source Catalog. Although it is possible that a strong evolutionary trend could be detected with such a modest redshift range, ideally redshifts of order unity would be required to detect significant changes in number density or luminosity. Although such studies are feasible with the largest radio telescopes, and of much interest in exploring the connection between ultra-luminous infrared galaxies and quasars, the depth of existing far-infrared surveys presently precludes the detection of such high-redshift OH megamasers. Nevertheless, the detection of IRAS20100-4156 implies that, for observations with Parkes and similar radio telescopes, the volume of space accessible for the study of luminous megamasers is greater than was previously thought by a factor of ~50.

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Detection of microvariability for BL Lacertae objects

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Large-amplitude, rapid optical variability is a well-known identifying characteristic for BL Lacertae objects ('blazars'). Although large-amplitude variations on timescales ranging from days to decades have been well documented[1], considerable controversy surrounds the nature of microvariability, that is, optical variations on timescales significantly shorter than a day. Here we report observations of BL Lacertae in which rapid changes were detected in the total optical flux from this object. These variations occurred on timescales as short as 1.5 hours. Although their structure is complex, the minimum timescale for the variations may be used to place constraints on the size of the emitting region. Assuming that these variations are produced in the vicinity of a supermassive black hole, the timescales imply a black-hole mass of $1.42\times10^9\,M_\odot$. Relativistic beaming need not be invoked to account for the present luminosity of this object.

Photoelectric photometry[2] of BL Lac suggested the possible existence of low-amplitude, short-term variations, but subsequent observations[3,4] failed to detect them. More recently, rapid changes have been observed[5,6] in the polarization for BL Lac on timescales significantly shorter than a day. These results have motivated our observational programme to study, using modern CCD (charge-coupled device) detectors, the nature and indeed the existence of microvariability for a selected sample of blazars. Here we report the first results of this study for the prototype of this class of objects, BL Lac.

The observations reported here were obtained with the No.1 0.9-m telescope at Kitt Peak National Observatory equipped with a direct CCD camera and an auto-guider. The observations were made through a V-filter with an RCA CCD. Repeated exposures of 90 s each were obtained of the star field containing BL Lac. Other stars on the frame provide comparisons for use in the reduction process. The observations were reduced using the method of Howell and Jacoby[7]. Each exposure is processed through an aperture-photometry routine which reduces the data as if it were produced by a multi-star photometer. Differential magnitudes can then be calculated for any pair of stars observed on the frame. Thus simultaneous observations of the object of interest and several comparison stars allows one to remove variations arising from fluctuations in atmospheric transmission and extinction.

The light curves for observations of BL Lac obtained on 11 and 14 July 1987 are shown in Figs 1 and 2 respectively. The upper panel in each figure displays the differential magnitude between comparison star 2 and BL Lac; the lower panel displays the differential magnitude between comparison star 2 and com-

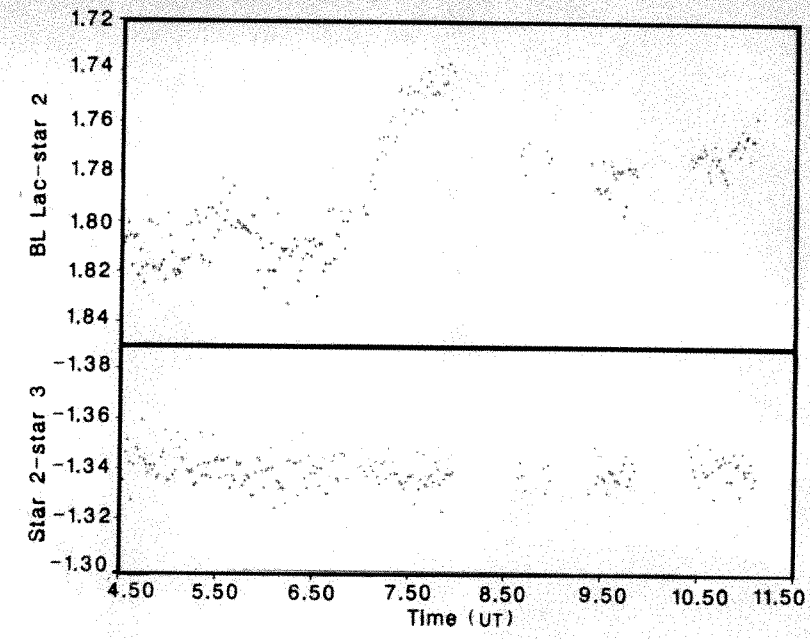


Fig. 1 Top, Differential magnitude between the variable BL Lac and comparison star 2 for the night of 11 July 1987. Bottom, Differential magnitude for comparison star 2 and comparison star 3 for the same night.

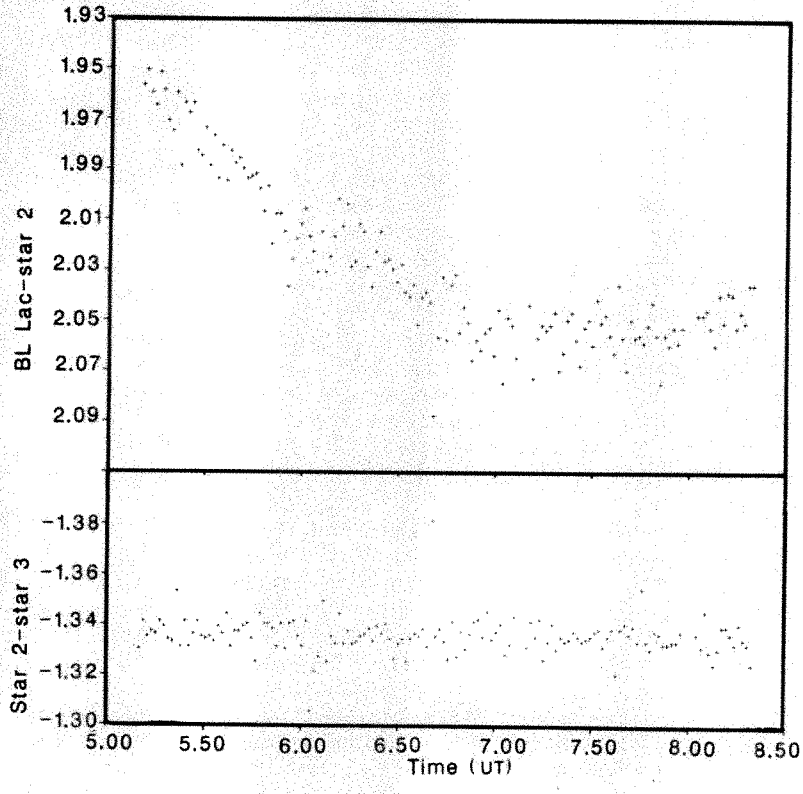


Fig. 2 Top, Differential magnitude BL Lac and comparison star 2 for the night of 14 July 1987. Bottom, Differential magnitude for comparison star 2 and comparison star 3 for the same night.

parison star 3. In each case, the scatter of the differential magnitudes in the lower panel provides an indication of the stability of the standard stars used in reducing these observations, and of the overall observational uncertainty.

On 11 July 1987 (Fig. 1) BL Lac was observed continuously for ~7 hours. From 04:30 to 06:30 UT, the source exhibited only mild variations, with a range of ~0.05 mag, and no conspicuous trends. From 06:30 to 08:00 UT, however, the microvariability of BL Lac could be characterized by a pronounced linear rise in brightness with a total change of ~0.12 mag. The source faded subsequently by ~0.04 mag, and exhibited no significant trends during the last three hours of the night.

On the night of 14 July 1987 (Fig. 2), the microvariability of BL Lac exhibited a decline of ~0.14 mag from 05:00 UT to 07:00 UT, but no systematic trends in brightness variations during the remainder of the night.

On 11 July 1987, when the microvariability exhibited a relatively complicated structure, BL Lac was near a brightness of $V = 15.0$ mag. This is near the middle of the range in brightness exhibited by the source in the past. It was slightly fainter (~0.25 mag) on 14 July 1987, when the steady decline in brightness was observed.

From a power-spectrum analysis of the time series on each of the nights we find no evidence for periodic modulations or the variations such as those reported for the blazar OJ287.

If one assumes that the microvariability detected for this object is generated in the vicinity of a supermassive black hole one can estimate the size of the emitting region as $R =$

assuming no relativistic beaming. The timescale identified from the present observations is the time during which the dramatic 0.12 mag. variation occurred on 11 July 1987. The duration of this event is approximately 1.5 hours. If one uses the timescale of this event to define a 'characteristic timescale' and one assumes that the radiation is generated close to the black hole, for example $R = 3R_s$ where $R_s = 2GM/c^2$ is the Schwarzschild radius, then the mass of the supermassive black hole, M, is given by $M = c^3 t/6G = 1.42 \times 10^9\ M_\odot$. The Eddington luminosity for a black hole of mass M is given by[9] $L_E = 1.3 \times 10^{38}\ (M/M_\odot)$ erg s^{-1} which in this case yields $L_E = 1.85 \times 10^{47}$ erg s^{-1}. For a brightness of ~15.0 mag, the luminosity for BL Lac is of the order of $L_v \approx 10^{45}$ erg s^{-1}.

Thus we find that although microvariability is definitely present on very short timescales, which in turn suggests a very small source region, the luminosity of BL Lac is significantly less than the Eddington luminosity, and does not require relativistic beaming for its explanation.

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A Solar System dust ring with the Earth as its shepherd

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Bodies orbiting in the gravitational fields of galactic, solar or planetary systems often suffer dissipative forces, including tidal interactions and drag resulting from motion through a gas or from collisions with dust grains. In the early solar nebula, gas drag induces resonance trapping, which may be of importance in the early accretional growth of planets[1–3]. By means of numerical integrations, we show here that small dust grains can be temporarily captured into exterior orbit–orbit resonances with the Earth, lasting from less than 10,000 years to more than 100,000 years. Grains with radii of 30–100 μm, orbiting in planes less than 10° from the plane of the Solar System and with orbital eccentricities of less than 0.3, are captured most easily. We argue that there should be an approximately toroidal cloud of particles, derived mostly from the asteroid belt, trapped into a variety of these exterior resonances. The cloud is mostly beyond the Earth's orbit, but includes it.

Three factors primarily motivated the present study. (1) The narrow bands observed along the ecliptic in the IRAS (Infrared Astronomical Satellite) data clearly seem to originate from dust particles in the main asteroid belt[4,5]; indeed, asteroid collisions may provide most of the dust grains that give rise to the entire broad band of observed zodiacal thermal emission[6]. (2) Grains smaller than ~100 μm in radius should successfully survive collisional destruction[7] to spiral in, under Poynting-Robertson[8] (P-R) and solar-wind drag, to well within the orbits of the Earth and Venus. (3) Venus and the Earth significantly perturb[9,10] the orbits (which are shrinking under P-R drag) of dust grains that pass these planets.

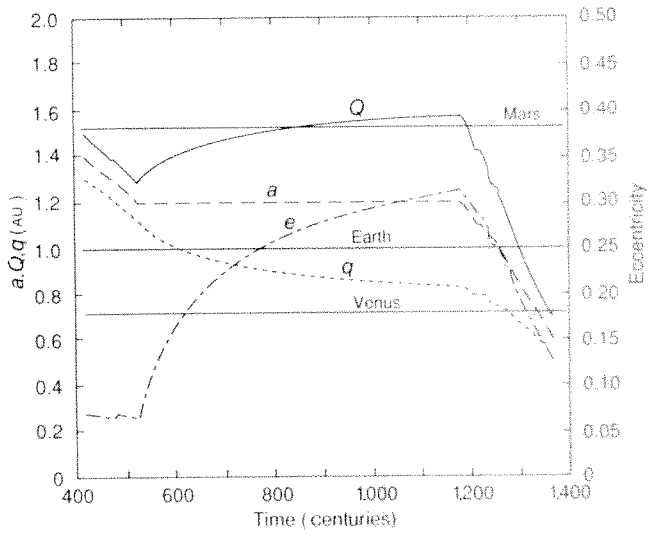


Fig. 1 Semi-major axis, a, aphelion distance, Q, perhelion distance, q, and eccentricity, e, as functions of time (in centuries) for a 30-μm radius particle started in an orbit with $a = 2.0$, $e = 0.1$ and an orbital inclination of 2.0°.

Some effects from dissipative forces in the Solar System have been noted. Gas drag, for example, can trap small bodies into $j:k$ orbital-period commensurabilities with a massive perturbing body (such as an accreting planet); here j and k are integers. Orbit-period commensurabilities of the type $j:j+1$ are often observed. Gonczi *et al.*[11] noted that, under P-R drag, small dust grains that were initiated in orbits at the interior 2:1 resonance with Jupiter could remain trapped there for some time; they did not, however, find any evidence for dust grains drifting, under P-R drag, into that resonance and becoming trapped.

Although our conclusions are consistent with those of Gonczi *et al.*, we also obtain new and different results (reported earlier in preliminary form[12]). These new results include long-term trapping (for more than 100,000 years) into resonances exterior to the Earth's orbit, short-term trapping (for typically less than 10,000 years) into resonances exterior to the orbits of Mars and Venus, and some limited trapping into interior resonances. For calculations we used Cowell integration in cartesian coordinates using the Everhart[13] implicit Runge-Kutta integrator with Gauss-Radau spacings. Radiation pressure is included and opposes the effect of solar gravity, with the assumption that particles behave as spherical black bodies of density 1 g cm^{-3}. The only other forces included are P-R drag and solar-wind drag, with the latter assumed to be 30% of the former. Solar-wind drag is included by multiplying by 1.3 the vector velocity term given by equation (5) in Burns *et al.*[14]. Many simulation runs incorporated the effect on the particles of the gravitational fields of the Sun, Venus, the Earth, Mars and Jupiter. To reduce computation, however, most runs included only the gravitational fields of the Sun, Venus and the Earth, and many were done with only those of the Sun and the Earth.

The trapping dynamics are best illustrated for the simplest case in which only the Sun, the Earth and a dust grain are involved. This case is shown in Fig. 1, where the orbital evolution of a particle of radius 30 μm is initiated with a semi-major axis, a, of 2.0 AU, an orbital eccentricity, e, of 0.1, and an orbital inclination of 2.0°. The plot starts at 40,000 years into the simulation. For the first 52,000 years, a decreases on average, under P-R and solar-wind drag, at a rate of 7.3 cm s^{-1}. The particle is then trapped into an external 3:4 resonance with the Earth at $a = 1.2037$ AU for the next 65,000 years; during this trapping period, a remains approximately constant, e increases, the perihelion distance, q, decreases to less than that of the

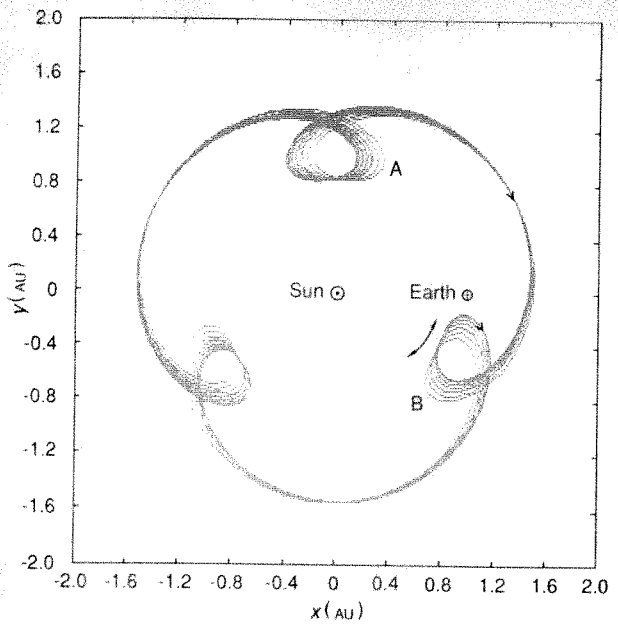


Fig. 2 The motion of the dust grain in Fig. 1, in a heliocentric coordinate frame rotating with the Earth. Shown here is the trajectory for 50 years of motion, projected onto the ecliptic, starting at 90,000 years into the simulation. The orbital motion tends to repeat itself in 220 years during quasi-stable trapping into the 3 : 4 external resonance with the Earth.

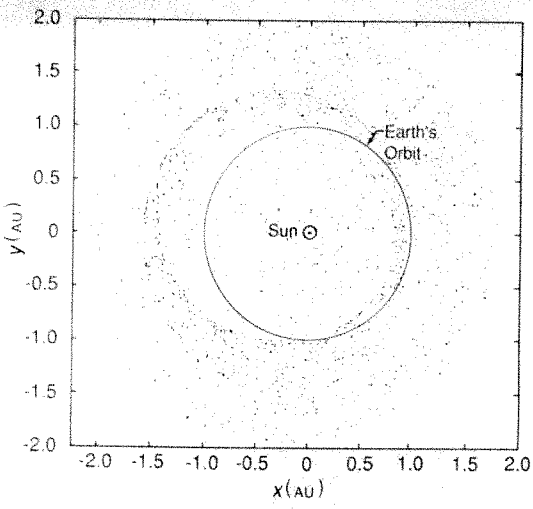


Fig. 3 The position of the particle for the orbit in Fig. 1, projected onto the ecliptic plane as a point approximately every 93 years as it evolves under gravitational and drag forces. The density enhancement arising from shepherding by the Earth is clearly evident.

Earth, and the aphelion distance, Q, increases. This trapping resonance is by no means unique: depending on the initial dust-grain orbital parameters, we have observed trapping in essentially all $j:j+1$ external resonances with the Earth from 2:3 to 13:14 (the latter for an initially circular orbit inclined at 10° to the ecliptic). We have also observed trapping into the 3:5 and 1:1 resonances. The 1:1 resonance resulted from the trapping of a 100-μm radius dust grain released from Comet Encke into a 'circulator' (rather than a horseshoe-shaped 'librator') resonance with the Earth.

Insight into the dynamics of the trapping can be obtained by transforming to a Sun-centred coordinate system rotating with the Earth's orbital motion. Figure 2 depicts particle motion, as viewed from ecliptic north, in such a coordinate system for 50 years, starting at 90,000 years into the same run as shown in Fig. 1. The Earth is shown to the right in this plot. At aphelion, the dust grain orbits more slowly around the Sun than does the Earth, and so moves in a clockwise fashion in this diagram. At perihelion, dust-grain orbital motion is faster than that of the Earth, and the grain moves in an anticlockwise direction. This pattern of motion gives rise to the three loops that are made during the four years that the particle takes to approximately retrace its path in this 3:4 resonance. The particle does not, however, exactly retrace its path on each orbit, because it is seldom precisely in the 3:4 resonance but only near it, in a quasi-stable trajectory, during the 65,000 years that it remains trapped. When the particle approaches relatively near, but trails orbitally behind, the Earth, just after particle perihelion passage, it receives a boost in orbital energy which increases its orbital semi-major axis a. If a becomes larger than the semi-major axis appropriate for the 3:4 resonance, then the dust grain has not quite completed three orbits by the time that the Earth has made four orbits. This causes the loops in the dust grain's orbit (Fig. 2) to drift in a clockwise direction. If this drift continues far enough, the gravitational downward 'kick' in the orbital energy of the dust grain can exceed the upward kick, because the dust grain now approaches closer to the Earth in loop A than in loop B. The orbital energy of the dust grain then decreases under both the Earth's gravitational force and the P–R and solar-wind drag forces until a becomes smaller than the resonance value. The loops then drift in an anticlockwise direction, and so on in a cyclic fashion. A high-resolution plot of semi-major axis against time clearly shows that a varies continuously throughout the trapping period to values both above and below the exact 3:4 resonance value of $a = 1.2037$.

In Fig. 3, we show the position of the particle projected as a point onto the ecliptic plane every 93.4377 years. It is clearly seen that the trapping phenomenon leads to an increased particle density near, but mostly outside of, a distance of 1 AU. The particle density is not uniform in heliocentric longitude because the line of apsides of the particle's orbit rotates by only ~180°, non-uniformly in time, during this particular trapping event. The orbital inclination of the particle in Figs 1, 2 and 3 remained near 2° throughout the trapping period, following which it varied rapidly to ~8°.

Large grains are trapped more easily into one of the external resonances than are small grains. Our numerical experiments have not generated a resonance with Mars for particle radii less than 60 μm. Large grains also tend to be trapped into resonances that have larger semi-major axes than those of small grains, and they stay trapped for longer periods of time. This can be explained by the relative rapidity with which small grains progress through a resonance trapping zone, a condition already noted for other types of trapping[15]. Grains with radii less than 10 μm are seldom trapped by the Earth or Venus and, when trapped, remain so only for very short periods. Grains of radius 30 μm are typically trapped exterior to the Earth for time spans of ~30,000 years, although they occasionally escape trapping altogether (even for orbits of low e and inclination). The overal physical process may be described as the 'shepherding' of dus grains into external orbit–orbit resonances by the combine perturbations of the Earth's gravity and P–R and solar-win forces. Mars is a less effective trapper because of its small mas and Venus loses trapping effectiveness primarily because gravitational disturbances from the Earth.

The question now arises as to whether or not any observation evidence exists for the dust ring that we predict near the or of the Earth. There are two sets of observations that are co sistent with, but not definitive proof of, a near-Earth dust ri The first of these is the detection of micrometre- to sub-micro etre-sized meteoroids by the Pioneer 8 and 9 spacecraft. Zoo has suggested that these could be created by collisions i

population of larger parent meteoroids whose spatial density increases with increasing heliocentric distance near 1 AU. McDonnell *et al.*[17], on the other hand, showed that the measurements could also be interpreted on the basis of a spatial density varying with heliocentric longitude. Which interpretation is correct should be resolved with the dust sensor on the Galileo spacecraft scheduled for launch in 1989.

The second set comprise zodiacal-light observations. In an examination of the zodiacal-light data gathered by the Helios spacecraft, Leinert *et al.*[18] failed to find the spatial-density increase suggested by Zook[16]. It is possible, however, that a low-intensity narrow ring near the Earth would not be easily detected by the photometers on Helios, because they did not make observations along the ecliptic plane. On the other hand, it may be significant that the spatial density of particles derived from zodiacal-light studies varies as $r^{-1.3}$ inside a radius of 1 AU (ref. 19) but as $r^{-1.5}$ or higher inverse powers[20,21] outside of this. Such a result could be caused by a dust ring near the Earth.

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Detection of methyl, hydroxymethyl and hydroxyethyl hydroperoxides in air and precipitation

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It is well established that organic peroxides are formed by OH-radical-induced oxidation of hydrocarbons under atmospheric conditions[1]. Peroxyacyl nitrates have been known to be constituents of polluted air since the 1950s[2,3]. In a recent study we have shown that the gas-phase reaction of ozone with a variety of natural and anthropogenic alkenes can contribute to the formation of hydrophilic organic peroxides[4]. Indications that such peroxides are actually present in the environment have been obtained previously by measurements of the peroxide content of cloudwater and rain. In the absence of a specific analytical method the peroxide content after selective enzymatic destruction of the hydrogen peroxide was taken to be the organic peroxide fraction[5–7]. In this letter we report the determination by high-performance liquid chromatography of methyl (MHP; CH_3OOH), hydroxymethyl (HMP; $HOCH_2OOH$) and 1-hydroxyethyl (HEP; $CH_3CH(OH)OOH$) hydroperoxides, in addition to H_2O_2, and present some preliminary concentration ranges in air and precipitation. The existence of this class of atmospheric trace constituents raises questions about possible adverse biological effects.

Although reversed-phase high-performance liquid chromatography (HPLC) is one of the most important methods of separating polar compounds in aqueous solution, it has been little used in the search for products of tropospheric photooxidation. It is superior to gas chromatography in the detection of thermally unstable substances. The method described here (Fig. 1) exploits the stability of hydroxyalkyl hydroperoxides in cold acidic solution by using HPLC to separate them from H_2O_2, which is normally present in much higher concentrations. Directly after separation, both the temperature and the pH of the eluate are increased to convert the hydroxyalkyl hydroperoxides to H_2O_2 and the corresponding aldehydes. H_2O_2 is then detected by the highly specific and sensitive *p*-hydroxyphenyl ethanoic acid/peroxidase fluorescence reaction[8]. Any H_2O_2 initially present in the sample is eluted first from the HPLC column and is detected directly. MHP is also detected directly; it is stable to the higher temperature and pH, but reacts in the presence of horseradish peroxidase.

The peroxides were identified by comparison of their retention times with those of standards (Fig. 2 and Table 1) or by admixture of standards to the samples. The limit of detection is 0.07 μmol l^{-1}, corresponding to 1.4×10^{-6} μmol per 20 μl of

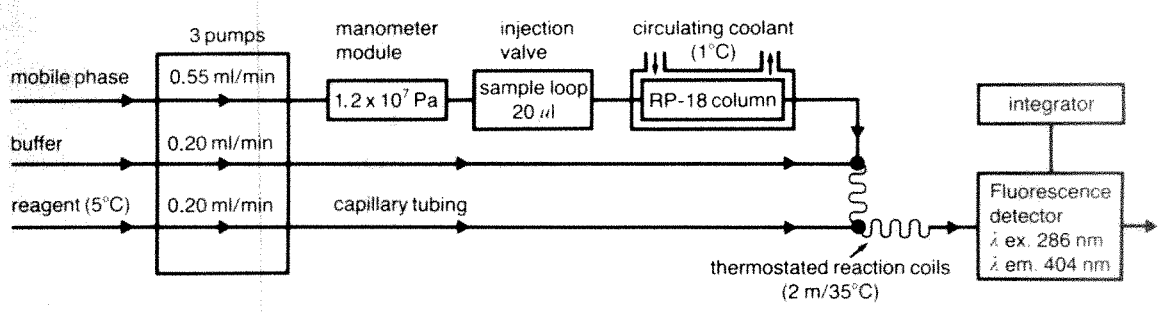


Fig. 1 Analytical system. HPLC: ABIMED, 3 Units Gilson model 302, 5 SC, Manometer module Gilson 802 C. Capillary tubing: o.d. 1/16", i.d. 0.12 mm. Injection valve: Rheodyne 7125. Cryostat: mgw Lauda K2R. Column: Bischoff Type NCi, 4 × 250 mm. Packing: 5-μm Shandon ODS Hypersil. Detector: Hewlett Packard HP 1046 A. Integrator: Hewlett Packard HP 3390. Water: deionized, distilled from $KMnO_4$, freed from traces of H_2O_2 with catalase, and redistilled. Mobile phase: diluted H_3PO_4, pH 3.5. Buffer: 0.01 M KH_2PO_4/NaOH, pH 9.4. Reagent: 0.5 mg *p*-hydroxyphenylethanoic acid (Merck) and 2 mg horseradish peroxidase (EC 1.11.1.7, ~170 U per mg, Merck) in 100 ml 0.01 M KH_2PO_4; 2 mg *p*-hydroxyphenylethanoic acid at peroxide concentrations > 10 μmol l^{-1}. At concentrations > 50 μmol l^{-1} the sample is diluted.

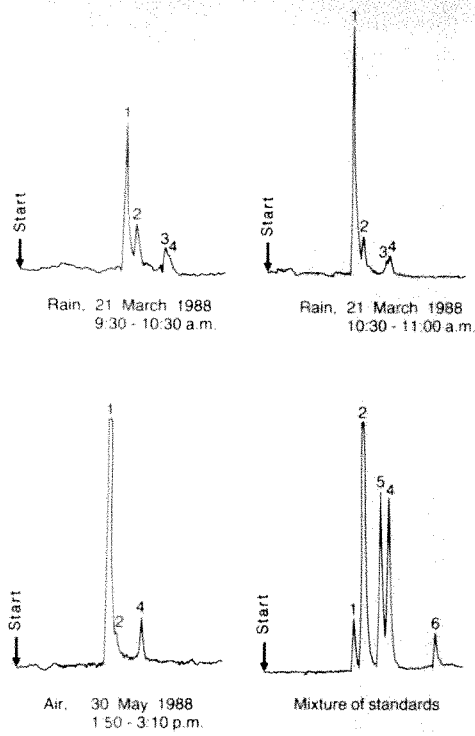


Fig. 2 Typical chromatograms (samples taken at Freising/Munich). See Table 1 for identification of peaks.

sample. Quantification was based on standard solutions of H_2O_2, MHP and bis(hydroxymethyl)peroxide (BHMP; $HOCH_2OOCH_2OH$) at a concentration of 1 mmol l^{-1} (iodometric titration). Calibration curves for freshly prepared equimolar dilutions of these were identical up to 10 μmol l^{-1}. HMP and HEP were not available as pure compounds, but always contained small amounts of H_2O_2 and the corresponding bis(hydroxyalkyl)peroxides, so that the calibration was only approximate. The linear range for H_2O_2 can be extended to 50 μmol l^{-1} by raising the concentration of *p*-hydroxyphenyl ethanoic acid in the reagent solution, but this is insufficient to extend the linear range for MHP beyond 10 μmol l^{-1}. Increased reagent also leads to a higher noise level and thereby to a loss in sensitivity. Because of the small difference in retention time ($\Delta R_t = 0.53$ min), the detection of HMP is difficult in the presence of much larger amounts of H_2O_2 (ratios of H_2O_2:HMP > 100:1). HEP and MHP, which have similar concentration ranges in natural samples, are also separated poorly from each other ($\Delta R_t = 0.20$ min). The rapid decomposition of HEP at pH > 7 means, however, that the difference between the peak areas measured in acidic and alkaline solution can be used for the quantification of these two peroxides.

During the period from the beginning of February to the end of March 1988, samples of snow from Freising/Munich had an average H_2O_2 concentration of 0.65 μmol l^{-1}. The highest concentration, 5.5 μmol l^{-1}, was measured on 24 February in a sample of wet snow taken around noon. In 87 of 93 samples the H_2O_2 content exceeded 0.07 μmol l^{-1}. HMP could be found in amounts near the detection limit (concentrations $c \approx 0.1$ μmol l^{-1}) in 11 samples.

Rain samples from the same period had significantly higher H_2O_2 concentrations (mean concentration $\bar{c} = 3.5$ μmol l^{-1}; maximum, $c_{max} = 14$ μmol l^{-1}). In 30 of 75 samples HMP was found at an average concentration of 0.1 μmol l^{-1}. Higher concentrations of HMP ($c_{max} = 0.6$ μmol l^{-1}) were more common in those rain samples having pH < 5.5 (compare data for 21 March 1988 in Table 2); HEP ($c_{max} = 0.3$ μmol l^{-1}), in contrast, was usually found in concentrations near the detection limit and, like MHP ($\bar{c} = 0.1$ μmol l^{-1}; $c_{max} = 0.3$ μmol l^{-1}), seemed to be nearly independent of pH in the range 4.4–6.1.

The peroxide concentration in 32 rain samples taken in April and May 1988 varied much more widely; for example, only 0.5 μmol l^{-1} H_2O_2 was found in water from a heavy shower at 1 p.m. on 20 May, whereas a light drizzle around noon on 17 May contained 110 μmol l^{-1}. HMP and HEP were not detectable on 16 and 17 May 1988 (Table 2); this can undoubtedly be attributed to the high pH (6.4–7.4) of the samples. In acidic rain (pH < 5.5) the relative concentrations of H_2O_2, HMP, HEP and MHP were typically 10, 0.7, 0.1 and 0.2 respectively; HMP, HEP and MHP attained maximum concentrations of 1.1, 0.3 and 0.5 μmol l^{-1} respectively. BHMP and perethanoic acid, though readily detected by the present method, have not been found in precipitation.

After extended periods of fine weather the first rain, typically with high pH values, contained little H_2O_2, no HMP or HEP and average amounts of MHP. One or two hours later, however, peak concentrations of H_2O_2 were reached at a pH that was still relatively high. Only as the pH dropped, with prolonged rain, could hydroxyalkyl hydroperoxides be found, along with average amounts of H_2O_2.

Analyses of 17 aqueous samples obtained by freezing water out of the air at −70 °C (Table 3) also attest to the presence of MHP; higher concentrations tend to occur before noon (c_{max} = 179 parts per 10^{12} by volume). As is to be expected from the higher pH(> 7), HMP and HEP could not be detected in samples taken in the morning. HMP could be found in some samples with pH ≈ 6 ($c_{max} = 15$ parts per 10^{12} by volume), but only if the analyses were carried out immediately after thawing.

The total peak area of the peroxides observed in particularly acidic rain samples was often less than that of the H_2O_2 peak found after making the sample basic. This indicates that part of the H_2O_2 is initially present in the form of other, as yet undetected, peroxidic compounds. Re-analysis of rain samples that had been stored frozen often gave irregular results (mostly indicating lower peroxide content). Thus, peroxide concentrations should be measured only in fresh samples, and the pH must always be taken into account.

Table 1 Identification of chromatogram peaks

Peak*	Identity	$\bar{R}_t$	Source of standard
1	H_2O_2	5.14 min	Perhydrol (Merck, Darmstadt), 35%
2	$HOCH_2OOH$ (HMP)	5.67 min	For synthesis and properties, see ref. 9.
3	$CH_3CH(OH)OOH$ (HEP)	6.98 min	Synthesis analogous to HMP; ^{13}C-NMR (D_2O/dioxane) $\delta = 21.0$ (q) 101.0 (d).
4	CH_3OOH (MHP)	7.18 min	For synthesis see ref. 10. ^{13}C-NMR (D_2O/dioxane) $\delta = 67.8$ (q); ^{1}H-NMR (D_2O/CH_3COOH) $\delta = 3.85$ (s).
5	$HOCH_2OOCH_2OH$ (BHMP)	6.69 min	For synthesis and properties, see ref. 11.
6	$CH_3C(O)OOH$	9.63 min	Peroxyethanoic acid (Peroxid-Chemie, München) 40%.

* See Fig. 2.

Table 2 Determination of H_2O_2, methyl, hydroxymethyl and hydroxyethyl hydroperoxides in rain water

Date/time		pH	Concentration in μmol l^{-1}			
			H_2O_2	$HOCH_2OOH$	$CH_3CH(OH)OOH$	CH_3OOH
21 March 1988*	8:00–9:00 a.m.	4.7	5.3	0.3	0.1	0.2
	9:30–10:30 a.m.	4.8	2.3	0.4	0.3	0.2
	10:30–11:00 a.m.	5.4	5.8	0.6	0.2	0.3
	12:15 p.m.‡	6.0	8.6	0.2	ND	0.1
	12:30 p.m.	5.9	7.0	0.2	0.1	0.2
	1:00 p.m.	6.1	5.8	0.2	0.1	0.1
	1:30 p.m.	5.9	5.3	0.4	0.1	0.1
	2:15 p.m.	5.1	6.3	0.5	0.1	0.1
	3:00 p.m.	4.7	6.0	0.6	0.1	0.1
	3:15 p.m.	4.8	6.0	0.5	0.1	0.2
	3:30 p.m.	4.4	6.8	0.6	0.1	0.2
	4:00 p.m.	4.4	6.7	0.5	0.1	0.1
16 May 1988†	**4:15 p.m.**	**7.3**	**9.0**	**ND**	**ND**	**0.2**
	4:45 p.m.	**7.4**	**9.3**	**ND**	**ND**	**0.1**
	5:00 p.m.	**7.0**	**35.2**	**ND**	**ND**	**0.3**
	5:30 p.m.	**6.7**	**63.1**	**ND**	**ND**	**0.3**
17 May 1988†	10:15 a.m.	7.1	20.0	ND	ND	0.3
	11:25 a.m.	7.0	57.4	ND	ND	0.3
	12:30 p.m.	6.4	110.6	ND	ND	0.4
	1:30 p.m.	6.4	28.8	ND	ND	0.1
19 May 1988*	**1:30 p.m.**	**4.4**	**36.2**	**>0.8§**	**0.3**	**0.5**
	1:45 p.m.	**4.2**	**57.1**	**>0.8§**	**0.3**	**0.5**
	3:25 p.m.	**4.1**	**32.4**	**>0.8§**	**0.3**	**0.3**

Rain at Freising/Munich was caught by a glass funnel (30 cm diameter) during the reported time period or until at least 0.5 ml was collected. The samples were analysed immediately. ND, not detectable.

* Sampling during an extended rain period.

† Sampling at the beginning of a rain period.

‡ Shoulder of the H_2O_2 peak, not precisely quantified.

Table 3 Determination of H_2O_2, methyl and hydroxymethyl hydroperoxides in air

Date/time		Air temperature (°C)	Relative humidity (%)	Sample weight (g)	Sample pH	Concentration (parts per 10^{12} by volume)		
						H_2O_2	$HOCH_2OOH$	CH_3OOH
30 May 1988	9:50–11:10 a.m.	15	61	3.8	7.4	453	ND	179
	12:05–1:25 p.m.	17	61	3.9	6.1	1,471	4*	73
	1:50–3:10 p.m.	18	59	3.9	6.1	914	4*	77
	3:30–4:50 p.m.	17	60	4.0	6.0	455	15*	40

0.5 m^3 air at Freising/Munich (1.2 m above ground) was drawn at 6.25 l min^{-1} with a gas sampler (Desaga GS 312) through an efficient cold trap (Brand) at −70 °C; the frozen sample was gently thawed and analysed immediately. ND, not detectable.

* Shoulder of the H_2O_2 peak, not precisely quantified.

In general, peroxide concentrations increased significantly whenever snow turned into rain. It is possible that snowflakes are less capable than raindrops of taking up peroxides while falling or that decomposition reactions occur on the ice crystals, as was observed in the analysis of frozen rain samples. The latter effect might reduce the peroxide content of samples frozen out of air. The analysis of these samples is further complicated by the possibility that some peroxides are not formed in significant amounts until the precursors are concentrated in the cold trap.

The range and the variation with time of the H_2O_2 concentrations in the present study are comparable with those reported by others[5,7,12–14]. In addition, the sum of the organic peroxide concentrations, on average ~1–10% of the H_2O_2 concentration, is in agreement with the values of Kelly *et al.*[5] and Lazrus *et al.*[7]. The occasional exceptionally high values of more than 30% organic peroxide in refs 5 and 7 were also found in very acidic rain samples. The absence of BHMP (the product of HMP and CH_2O in acidic solution) can be attributed to the low ratio of CH_2O to H_2O_2 in rain water. Perethanoic acid, on the other hand, which has been postulated as an intermediate in the atmospheric degradation of hydrocarbons[1,15], is too rapidly hydrolysed to H_2O_2 and ethanoic acid in dilute aqueous solution to be detected in rain samples by the present method. MHP has been found in simulated atmospheric oxidation of hydrocarbons such as methane and is predicted to be a trace constituent of the troposphere[16–18]. Its low concentration in the air on sunny afternoons and its occurrence in many of our rain samples appear to confirm that photolysis and washing out by rain are major sinks for this peroxide[19].

There are two plausible atmospheric pathways for the formation of hydroxyalkyl hydroperoxides. The main route may be the addition of H_2O_2 to aldehydes under acidic conditions. Of importance in this context is the fact that H_2O_2 creates its own acidic environment by oxidizing the ubiquitous atmospheric SO_2 to sulphate[20,21]. The alternative route to these peroxides involves the addition of water to the so-called Criegee intermediate, R_1R_2COO, of the reaction of ozone and alkenes[22] (R_1 and R_2 are alkyl groups or hydrogen); no acid is required for this addition, but the lifetime of the peroxides is of course far longer

if acid is present. The formation of hydroxyalkyl hydroperoxides was established in our earlier simulations of gas-phase ozonolysis of naturally occurring alkenes (for example, terpenes, isoprene and ethene)[4]. In a recent study[13], concentrations of H_2O_2 were sometimes found to be much higher in a forest than those just outside it, suggesting that some of the H_2O_2 may be formed by decomposition of hydroxyalkyl hydroperoxides produced in the rapid reaction of ozone with biogenic alkenes.

There are too few studies of the effects of the peroxides described here on intact organisms to justify any definite statements about their probable environmental significance. Various physiological effects of HMP have, however, been examined in detail[23,24]. Among these is the irreversible inhibition of horseradish peroxidase. Inhibition of plant peroxidases by low concentrations of HMP might greatly increase the toxic effects of H_2O_2, which have been found in experiments on trees[25]. Damage of a type attributable to peroxides has often been mentioned in descriptions of *Waldsterben* (forest death)[26], but simulations using environmentally relevant concentrations of ozone and peroxyacetyl nitrate, the usual representative photo-oxidants, have failed to reproduce all the symptoms of this damage[27,28]. These studies may have taken too little account of the possibility that the products of ozone, not ozone itself, are responsible for the damage to plants. If during the experiment the reaction products are not allowed to build up in the air surrounding the plants, as they probably do in nature, fumigation with ozone may not produce the effects observed in forests. A recent set of experiments on pea seedlings in a laboratory showed that ozone causes more damage when in combination with stress ethylene[29], a result which is consistent with our suggestion. Studies are urgently needed to determine to what extent and by what route terpene oxidation products, among them the peroxides discussed here, can penetrate plant tissues and what their effects on these tissues are. This work indicates that damage by hydroxyalkyl hydroperoxides should be greater in acidic environments. It is perhaps relevant that there are several reports that ozone and acidity act synergistically in damaging plants[30,31]

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Three-dimensional micromagnetic modelling of ferromagnetic domain structure

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The fidelity of magnetic recording materials (whether they be synthetic crystals on a recording tape, or natural crystals in rocks recording changes in the Earth's magnetic field) depends on the uniformity of their magnetic structure. Such structure is determined by minimizing the energies arising from atomic magnetic dipole interactions. Previous micromagnetic models of coupled spin structures have contained well defined constraints to make the calculations tractable. By using a supercomputer, we have been able to perform unconstrained calculations of minimum energy structures for cubic assemblies of up to $22\times22\times22$ exchange-coupled spins. The critical size for transformation from a uniform single-domain structure to a non-uniform three-dimensional structure is about 0.1 μm for magnetite, as found previously from one-dimensional modelling. However, a variety of different non-uniform structures are possible, with energies and magnetic moments much less than those of conventional lamellar domains. The predicted moments of unweighted combinations of these states agree well with experimental measurements on magnetite in the size range 0.08–0.5 μm. Surface spin structures are such as to minimize flux leakage out of the particle and might be misleadingly imaged by the Bitter colloid technique as indicating a single-domain state.

Early micromagnetic models of domains were analytic[1,2], and permitted only a one-dimensional variation in the spin angle between domains of uniform magnetization[3]. Computer techniques have facilitated numerical models that allow non-uniform solutions[4,5]. Although initially such models were one-dimensional, LaBonte[6] and more recently Aharoni and Jakubovics[7], Fredkin and Koehler[26], and Zhu and Bertram[27] have achieved two-dimensional solutions.

One- and two-dimensional solutions have proved useful in investigating the magnetic stability of ferromagnetic crystals[8-10], but there is no doubt that the true magnetization structure is three-dimensional. Here we describe a fully three-dimensional micromagnetic model, in which no constraints are placed on the direction of magnetization and the bodies considered are finite in all three dimensions.

For simplicity, we consider cubes of magnetite in zero external field at room temperature. Generalization to other temperatures, fields, crystal forms and phases is straightforward and will be described elsewhere. Each cube is divided into N^3 sub-cubes. The magnetic moment of any sub-cube is the average over a very large number of atomic spins and is represented by a dipole $\mathbf{m}_i$ at the centre of the sub-cube. All dipoles have the same magnitude m_i but their directions vary.

The contributions to the total magnetic energy of a cube were formulated as described by Brown[11]. (1) The exchange energy, E_E, is due to coupling of the spins $\mathbf{S}_i$ and $\mathbf{S}_j$ of nearest-neighbour atoms:

$$E_E = -2\sum_x\sum_y\sum_z J_E\mathbf{S}_i\cdot\mathbf{S}_j$$

where J_E is the exchange integral. As in other micromagnetic calculations[12], the J_{AB} (inter-sublattice) interaction is assumed to dominate. We also assume linear variations of the atomic spin directions in the x, y and z directions between the centres of the sub-cubes. (2) Magnetocrystalline anisotropy energy, E_A, is due to spin-lattice coupling:

$$E_A = \sum_x\sum_y\sum_z (K_1(\alpha_1^2\alpha_2^2+\alpha_2^2\alpha_3^2+\alpha_3^2\alpha_1^2)+K_2\alpha_1^2\alpha_2^2\alpha_3^2)$$

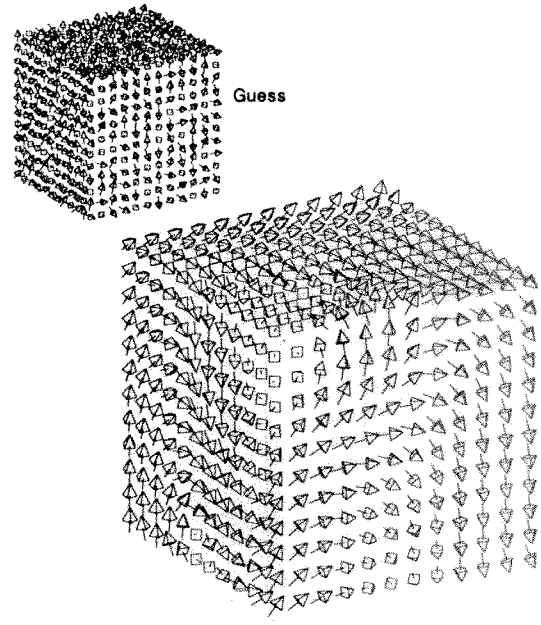


Fig. 1 A minimum-energy state of 12^3 exchange-coupled dipoles, obtained by an unconstrained minimization routine, starting from the initially guessed structure shown in the insert. The minimum-energy state involves no imposed symmetry from a random initial guess. Grain size = 0.2 μm cube. Reduced energy = 0.54. $M_{rs}/M_s = 0.28$.

where K_1, K_2 are the anisotropy constants and α_1, α_2, α_3 are the direction cosines of $\mathbf{m}_i$. (3) Magnetostrictive strain energy, E_S, is due to lattice deformation caused by the magnetization of the crystal:

$$E_S = \frac{3}{2}\sum_x\sum_y\sum_z \lambda\sigma\cos^2\phi$$

where λ is the magnetostriction constant, σ is the magnetoelastic stress and ϕ is the angle between the stress and magnetization direction. (4) Demagnetizing energy, E_D, is due to magnetostatic dipole-dipole interaction:

$$E_D = -\frac{1}{2}\sum_x\sum_y\sum_z \mathbf{m}_i \cdot \mathbf{H}_i$$

where $\mathbf{H}_i$ is the magnetic field at the location of $\mathbf{m}_i$ that results from all the other dipoles.

Stable magnetic structures were determined by finding minima of the sum $E_E + E_A + E_D$, using previously published values for the saturation magnetization[13], the anisotropy constant[14] and the exchange constant[15]. The magnetostrictive energy in magnetite is nearly two orders of magnitude smaller than any of the other energies involved and was not considered further in this initial study. The anisotropy of the solutions shown in Fig. 2 was simplified to a uniaxial anisotropy along the $+z$ direction. Solutions with cubic anisotropy, such as those in Fig. 1, were not significantly different, because of the dominance of magnetostatic energy which strongly favours alignment of spins parallel to crystal surfaces.

The localized energies E_E and E_A are easy to compute. E_D, however, is long-ranged and nonlinear. Even for a relatively low resolution of 12^3 sub-cubes, the demagnetizing energy requires ~1.5 million interaction calculations per iteration. The computation can be reduced considerably by rewriting the demagnetizing energy in the manner of Rhodes and Rowlands[16], as the product of an invariant demagnetizing coefficient and variable equivalent magnetic charges on the surfaces of the sub-cubes[10]. In one-dimensional models, only interaction coefficients between parallel sheets of magnetic charge are needed. For the three-dimensional problem, the interaction coefficients between orthogonal sheets must also be calculated.

The minimization was performed using the conjugate-gradient method on a Cray X-MP/22 supercomputer. Second-order methods converge faster but memory constraints did not allow storage of the hessian matrix. Figure 1 demonstrates the effectiveness of the minimization technique. This minimum-energy state was obtained in just under 50 minutes of computer time from a random initial guess.

Because of the large number of dipoles, numerous different minimum-energy states exist. To make the computations more efficient and allow us to increase the resolution to 22^3 sub-cubes, we next restricted our attention to symmetric solutions in which the structure in a given octant of the cube is either symmetric or antisymmetric with respect to that of a reference octant. Non-symmetric minimum-energy states (such as that in Fig. 1) always had higher energies than corresponding symmetric states with similar features (for example, Fig. 2*d*). All our solutions were tested for stability by removing the symmetry requirement and solving for a new minimum-energy structure.

Symmetric structures of various types are shown in Fig. 2. There are no lamellar domains of the classic Kittel[2] type. Regions of uniform magnetization correspond to areas of constant colours. These domains have dipoles aligned in the $\pm z$ directions. The net moments of the cubes are also aligned in this direction. In the single-domain (or type 1A) solution (Fig. 2*a*) the moments are not all exactly parallel, as in a classical single-domain grain; spins are deflected at the corners, where the demagnetizing field is strongest[17]. As the grain size increases the deflections at the corners become more pronounced. Above ~0.2 μm a new type of structure arises, in which the moments at the cube edges reverse to form domains aligned in the $-z$ direction (Fig. 2*b*). This solution (type 1B) is a five-domain grain, having four domains in the $-z$ direction at the cube edges and a core domain in the $+z$ direction.

A classical two-domain grain (type 2 solution) can also be generated (Fig. 2*c*) which has a lower energy than the single-domain or five-domain solutions in the grain size range 0.096–0.35 μm. Thus the critical single-domain size d_0 in magnetite is ~0.1 μm, as predicted by one-dimensional theories[8,10,15,18], and the equilibrium structure above d_0 is a two-domain state with large closure domains and zero net moment. The solution (type 3) that is energetically the most favourable above ~0.35 μm is shown in Fig. 2*d*; this also a five-domain grain but is characterized by curling of the magnetization throughout the body of the grain.

The energies of these structures are between 2 and 3 times lower than those of one-dimensional solutions in the same grain-size range. This reduction in energy is due to the fact that the spins are free to rotate and thus to reduce the surface magnetic poles. A similar reduction of the surface-pole density was noted by LaBonte[6] for two-dimensional solutions of 180° domain walls in thin films. The net effect is that spins near the

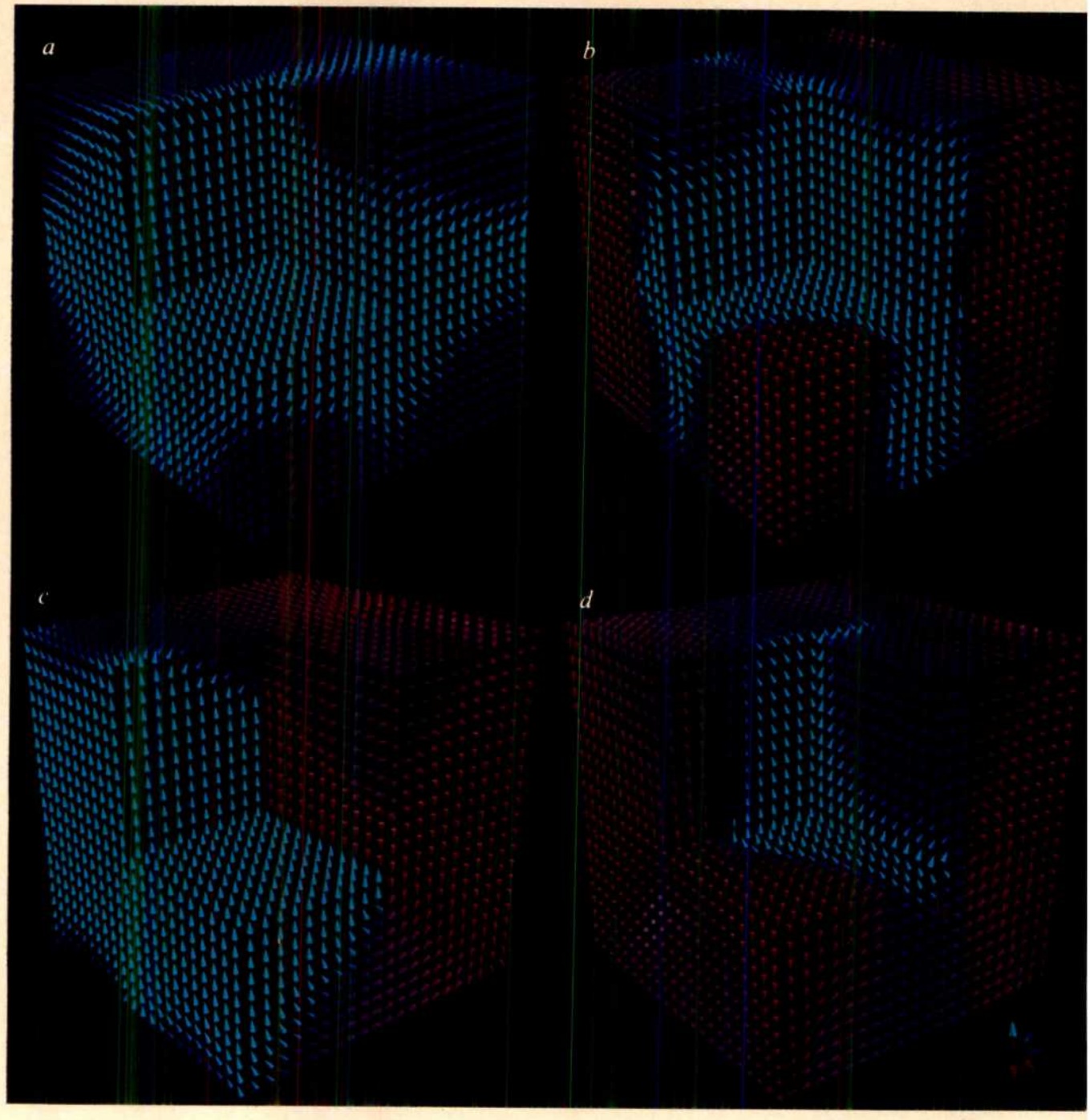


Fig. 2 Various types of symmetric solutions for 0.2 μm cube grain size. The solid cones representing the magnetic dipoles are coloured according to their angle from the vertical: light blue represents up and dark red represents down. *a*, A single-domain structure (type 1A), showing deflection of the magnetization at the cube corners. Reduced energy = 0.81; $M_{rs}/M_s = 0.89$. *b*, A five-domain structure (type 1B), obtained using the same symmetry as that of type 1A. Reduced energy = 0.78; $M_{rs}/M_s = 0.32$. *c*, A two-domain structure (type 2), with large closure domains. Reduced energy = 0.39; $M_{rs}/M_s = 0.0$. *d*, A five-domain structure (type 3) dominated by internal flux loops. Reduced energy = 0.50; $M_{rs}/M_s = 0.12$.

grain surface tend to lie parallel to the surface, so that Néel walls are favoured over Bloch walls, in which spins rotate in a plane perpendicular to the surface. Where Bloch walls do occur, as on the side faces of Fig. 2*b*, they are much narrower than predicted by one-dimensional models. Therefore Bloch walls themselves will not contribute significantly to the net magnetic moment of the grain.

We can compare our predictions of magnetic moments and Bitter patterns with experimental observations. Saturation remanence (M_{rs}) is a bulk magnetic parameter which has been measured for well-sized dispersed magnetite grains. It is likely that all the different domain configurations that are possible in a grain of a particular size will coexist and contribute to M_{rs}. Assuming equipartition of the permitted symmetric states, we predict the grain-size dependence of the reduced saturation remanence M_{rs}/M_s plotted in Fig. 3. A factor of 0.5 has been incorporated in the theoretical curve to allow for random orientations. This value is appropriate for crystals dominated by uniaxial shape anisotropy, as has shown to be the case[19,20] for many of the samples whose data appear in Fig. 3. There is good agreement with the published experimental data over the range 0.08–0.5 μm.

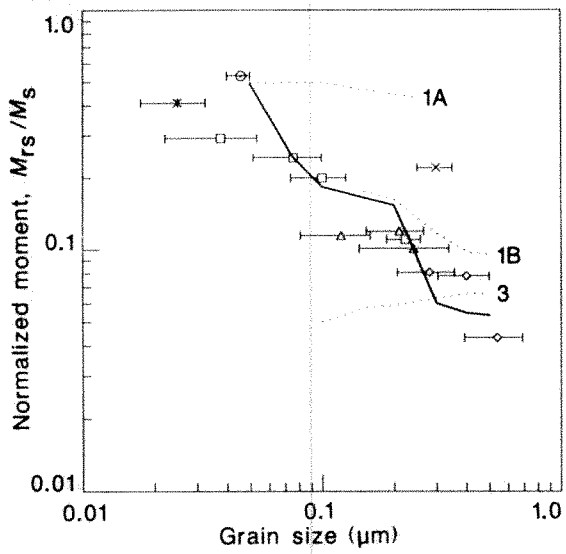


Fig. 3 Predicted grain-size dependence of reduced saturation remanence M_{rs}/M_s (solid curve) compared with experimental data. The dotted curves are the theoretical predicted values for the different structures shown in Fig. 2. Note that the two-domain structure (type 2) has zero net moment. The experimental data were obtained from: △ Levi and Merrill[20], □ Dunlop[21], * Özdemir and Banerjee[22], × Schmidbauer and Veitch[23], ⊙ Moskowitz *et al.*[24] and ◇ Dunlop and Argyle[25].

Bitter colloid patterns form in response to the magnetic field gradient normal to the surface of a grain. Because Néel walls do not generate large normal field gradients, even quite complex structures, such as that of Fig. 2*d*, might not be imaged by the Bitter technique, so that such a grain might be interpreted as comprising a single domain.

The results obtained so far show good agreement with bulk measurements of M_{rs}. Unfortunately we lack the experimental observations of Bitter patterns on submicrometre grains that are needed to test our predicted structures more rigorously. However, testable predictions of temperature and field dependencies of these structures are currently being investigated.

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Sensitivity of climate and atmospheric CO_2 to deep-ocean and shallow-ocean carbonate burial

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Previous models of physical and biogeochemical controls of the evolution of atmospheric CO_2 and climate over hundreds of millions of years have neglected the effect of variations in the balance between shallow-ocean and deep-ocean carbonate deposition. This is important because the relative proportions of carbonate burial in the two reservoirs have changed over time. Here I set up a model of the carbonate–silicate geochemical cycle that distinguishes carbonate masses produced by the two types of burial and shows that reasonable increases in deep-ocean burial could produce substantial warmings over a few hundred million years. The model includes exchanges between crust and mantle; transients from burial shifts are found to be sensitive to the fraction of non-degassed carbonates subducted into the mantle. Without the habitation of the open ocean by plankton such as foraminifera and coccolithophores, today's climate would be substantially colder.

Over geological timescales, atmospheric CO_2 (P_{atm}), and therefore global temperature (T), is controlled by differing balances of sources and sinks[1–7]. Models have examined, in varying degrees of detail, forcings upon and changes in these source and sink terms, including net organic-carbon burial[3], sea-floor generation rate and continental area[2,3], P_{atm} and T feedbacks in the weathering sink[1,2,5] (the chemical weathering of continental silicates, followed by carbonate burial), and the effect of the terrestrial biosphere[5,6]. Until now, models of the geochemical carbonate–silicate cycle that explicitly calculated P_{atm} have failed to resolve the different dynamics of pelagic and continental carbonates. The relative proportions of carbonate entering these two reservoirs have changed over time[8,9], and there is strong evidence that the proportion of pelagic burial has increased substantially since the middle Jurassic[9–11].

A fundamental division of carbonates into those deposited in shallow water and those deposited in deep water is required to examine how their different dynamics could affect atmospheric CO_2, primarily because, mass for mass, the metamorphic flux of CO_2 to the atmosphere is much larger from pelagic than from continental carbonates. Burial in shallow water produces carbonates that will eventually be weathered or degassed on the continents; these continental carbonates decay statistically at a rate that is slower by an order of magnitude than that of the pelagic carbonates[9]. Burial in deep water produces pelagic carbonates whose sink consists of the subducting slabs at plate boundaries[12]. There, under conditions of higher heat and pressure, they may either be metamorphosed to silicates, accompanied by outgassing of CO_2 which eventually enters the atmosphere, or remain as carbonates on the slabs as they are subducted into the mantle[13]. The cycle of carbon between the mantle and crust has been shown to be important in the geochemical carbon cycle[12–14], but has not yet been incorporated explicitly into models for P_{atm}.

Our model for P_{atm} and masses of continental (M_c) and pelagic (M_p) carbonates is illustrated in Fig. 1, with interconnecting fluxes of carbon, F. Because the organic-carbon cycle is of only secondary importance[3], the only sink for P_{atm} considered here is that associated with silicate weathering (F_{sw}). The weathering fluxes—F_{sw} and that from the continental carbonates, F_{cw}—are derived from their present-day values ($F_{sw,ref}$ and $F_{cw,ref}$) using a non-dimensional weathering-rate factor $f_{wr}(T)$ (ref. 2). The temperature dependence of f_{wr} (equation 2) incorporates effects directly related to T as well as the effect of

temperature on global runoff; other forms for weathering are available[1,5], but would not essentially alter the conclusions. As in ref. 2, the weathering formulations assume that silicate weathering is unlimited, but that M_c affects carbonate weathering (see equations 1a, b). A greenhouse equation[2] relates P_{atm} and T (equation 3). Metamorphic release of CO_2 from continental carbonates is assumed to be proportional to M_c, and for simplicity, the flux of pelagic carbonates entering the subduction zones is also assumed to be proportional to M_p, as in ref. 9 (equations 4a, b). In reality, there is a varying delay during spreading between carbonate deposition above the carbonate compensation depth on the ridges and its subduction several tens of millions of years later. This delay is neglected here as we are concerned only with the fundamental dynamics of the system. Therefore, our equations are

$$F_{sw} = F_{sw,ref} f_{wr} \quad ; \quad F_{cw} = F_{cw,ref} f_{wr} \frac{M_c}{M_{c,ref}} \qquad (1a, b)$$

$$f_{wr} = 1.0 + 8.7 \times 10^{-2}(T - T_{ref}) + 1.9 \times 10^{-3}(T - T_{ref})^2 \qquad (2)$$

$$T - T_{ref} = 2.88 \ln\left(\frac{P_{atm}}{P_{atm,ref}}\right) \qquad (3)$$

$$F_{cm} = k_{cm} M_c \quad ; \quad F_{sub} = k_{sub} M_p \qquad (4a, b)$$

We introduce a burial parameter β ($0 \le \beta \le 1$), representing the way in which physical and biogeochemical ocean processes proportionate the weathering fluxes between the pelagic carbonates, $\beta(F_{sw} + F_{cw})$, and the continental carbonates, $(1-\beta) \times (F_{sw} + F_{cw})$. Abiotic carbonate precipitation in the hydrothermal cooling systems of mid-ocean ridges[15] could also be considered to contribute to β. The parameter β is obviously the same for both silicate and carbonate weathering, because precipitation does not distinguish calcium ions derived from different rock types.

The subduction flux, F_{sub}, is divided, using a subduction parameter $\sigma (0 \le \sigma \le 1)$, into a flux entering the mantle, σF_{sub} and a flux representing metamorphic decarbonation $(1-\sigma)F_{sub}$, which produces a source flux of CO_2 to the atmosphere. Transfer from M_p to M_c during lateral accretion of pelagic sediments to a continent at a convergent plate boundary is assumed to be negligible in this context. Finally, the flux of outgassed CO_2 from the mantle entering the atmosphere from the ocean ridges, F_r, is assumed to be constant. The governing equations for the three reservoirs are then:

$$\text{for } M_c: \quad \frac{dM_c}{dt} = (1-\beta)F_{sw} - \beta F_{cw} - F_{cm} \qquad (5)$$

$$\text{for } M_p: \quad \frac{dM_p}{dt} = \beta(F_{sw} + F_{cw}) - F_{sub} \qquad (6)$$

$$\text{for } P_{atm}: \quad F_r + F_{cm} + (1-\sigma)F_{sub} - F_{sw} = 0 \qquad (7)$$

which control M_c, M_p and P_{atm} respectively. A steady-state balance for P_{atm} must be maintained in the ocean/atmosphere system at all times over the timescales in this problem[1,2,5,7], even though P_{atm} varies with time in the longer term.

Figure 2 shows the results of perturbing an initial steady-state. The initial values are broadly consistent with the relationships in refs 2, 9, and 13 (see figure caption). This state is supposed to represent neither the present, although it draws upon many of the relationships that exist therein, nor any particular point in the past. Rather, it serves as a generic condition from which to study the response-dynamics of the system.

We introduce a step increase to β, $\Delta\beta$, at time $t = 0$. In Fig. 2, a trio of runs with $\Delta\beta = 0.1$, 0.3 and 0.5 shows that global warming follows the step increase to a degree that increases with larger $\Delta\beta$. For all of these cases, which have $\sigma = 0.5$ (a possible upper bound[13]), T is nearly maximal between 100 and 200 Myr after the β-step. The peak warmings for the three cases,

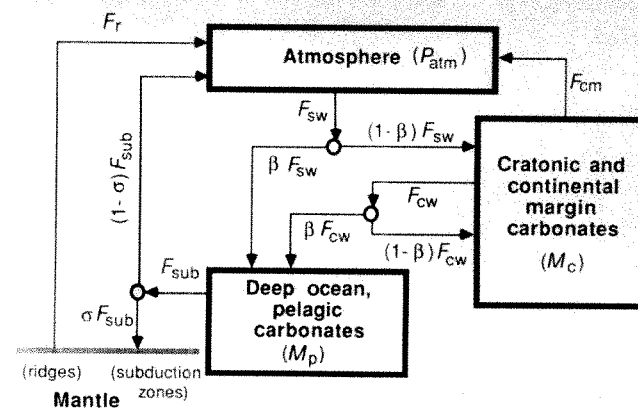


Fig. 1 Flows of carbon between reservoirs. The weathering fluxes associated with the weathering of silicates (F_{sw}) and continental carbonates (F_{cw}) are diverted by a burial parameter (β), which represents the degree to which physical and biological ocean processes deposit the carbon as carbonates either in shallow water, to become continental carbonates, or in deep water, as pelagic carbonates. The subduction flux (F_{sub}) either enters the mantle as carbonate or is metamorphosed, accompanied by CO_2 degassing into the atmosphere; this division is quantified with a subduction parameter (σ). The model also incorporates CO_2 fluxes to the atmosphere from the ocean ridges (F_r) and from continental metamorphism (F_{cm}).

respectively, are 2, 6 and 11 °C, and are followed by much slower cooling. These two trends—warming followed by cooling—derive from different rates of equilibration between sub-systems. In general, following the β-step, the increased burial flux to the deep ocean increases the pelagic carbonate mass, which increases CO_2 input to the atmosphere. Because of their relatively large mass, a decrease of only a few per cent in the mass of the continental carbonates causes a proportionately much larger increase to the pelagics. Over longer times, adjustment occurs between the total crustal carbonate system and the mantle, as the masses of both continental and pelagic carbonates decrease. In Fig. 2, as the pelagic mass declines, so does the metamorphic source flux of CO_2 to the atmosphere, hence giving rise to cooling. Note that the system is out of equilibrium for the Phanerozoic-length period of time shown in Fig. 2.

Because the present-day subduction efficiency σ is not well constrained (lying between 0 and 0.5 (ref. 13)), we have also examined the effect of varying this parameter. Using one case from the first trio of runs as a reference ($\Delta\beta = 0.1$, $\sigma = 0.5$), σ is decreased to 0.3 and 0.1. For a fixed $\Delta\beta$, a smaller σ gives a larger rate of warming, a larger peak ΔT and a longer time to reach the peak ΔT. For example, ΔT rises to ~8 °C by 400–500 Myr after the β-step, for $\sigma = 0.1$. This sensitivity arises because a smaller σ means that a higher metamorphic source flux of CO_2 to the atmosphere from the subduction zones is maintained for a given mass of pelagic carbonate. Figure 2 shows that substantial climatic changes follow even small shifts in β if σ is small, and also occur with larger values of σ if the shifts in β are larger.

The main objective of this study is to point out that partitioning of ocean carbonate burial must be incorporated in studies of the evolution of atmospheric CO_2 and climate. Evolutionary changes, such as the proliferation of coccolithophores after the Triassic and of globigerinacean foraminifera after the late Jurassic, shifted a significant portion of global carbonate deposition from the shelves to the deep ocean[8]. Wilkinson and Walker[9] propose that a gradual shift in β from about 0 to 0.6 has occurred over the past 140 Myr. (β might never have been zero, for hydrothermally induced carbonate precipitation at ocean ridges[15] may prevent β from falling below a certain minimum value. Also, even in the absence of $CaCO_3$-precipitating organisms in the open ocean, abiotic deep-ocean precipitation would have been required to maintain chemical balance if sea level

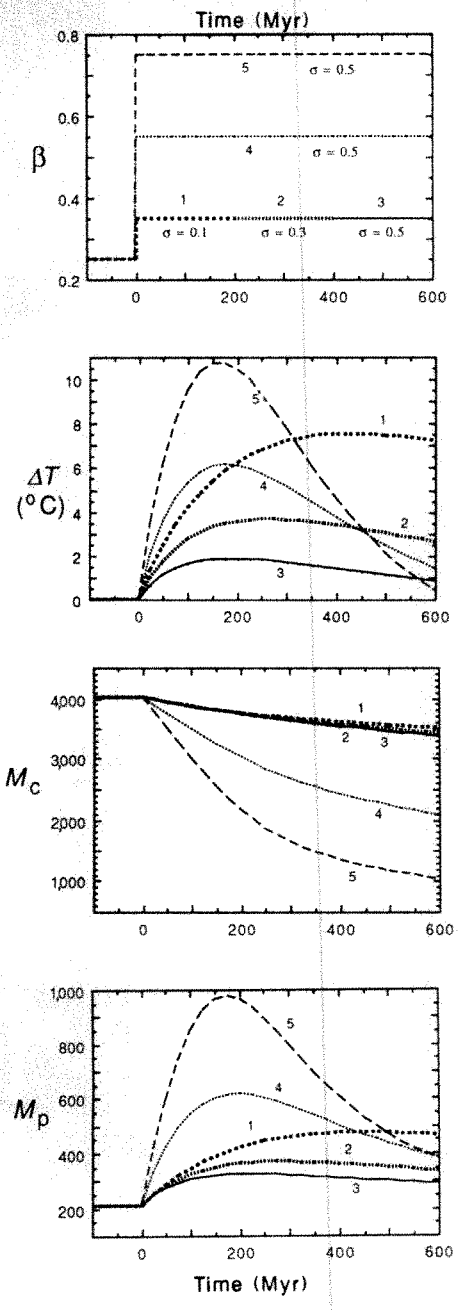


Fig. 2 Results of model runs, demonstrating the effect of a step increase, $\Delta\beta$, in the burial parameter β, for $\Delta\beta = 0.1$, 0.3 and 0.5 (cases 5, 4 and 3, with the subduction parameter $\sigma = 0.5$). To demonstrate sensitivity to σ, cases 3, 2 and 1 have $\sigma = 0.5$, 0.3 and 0.1, respectively, each with $\Delta\beta = 0.1$. All cases have identical initial values for β, T, M_c and M_p, and are initially in steady-state. These identical initial values can be set for different values of σ by varying F_r to satisfy $F_r = \sigma F_{sub}$, with F_{sub} taking the value for a steady-state solution with $\sigma = 0$. To satisfy the equations described in the text, the system requires reference values. We use those in ref. 2: $F_{sw,ref} = 9 \times 10^{18}$ mol myr^{-1}; $F_{cw,ref} = 12.5 \times 10^{18}$ mol myr^{-1}; $M_{c,ref} = 4{,}000 \times 10^{18}$ mol; $T_{ref} = 15$ °C; $P_{atm,ref} = 310$ μatm. Following ref. 9, $k_{sub} = 0.022$ myr^{-1} (approximately the inverse of the average age of ocean floor[19]). The value for k_{cm} is chosen such that $k_{cm} \ll k_{sub}$ and k_{cm} is less than the weathering rate constant of continental carbonates (~0.002 myr^{-1} in ref. 9) but is not negligible; we use $k_{cm} = 0.0005$ myr^{-1}. Somewhat arbitrarily setting $\beta = 0.25$ for the initial steady-state gives a reasonable crust and mantle silicate-carbonate cycle for perturbation studies, specifically, initial conditions of $M_c = 4{,}020 \times 10^{18}$ mol; $M_p = 210 \times 10^{18}$ mol (for present-day values[10], $M_p < 0.1$ M_c); $P_{atm} = 380$ μatm; $T = 15.6$ °C; $F_{cm} = 5.0 \times 10^{18}$ mol myr^{-1}; $F_{cw} = 10.6 \times 10^{18}$ mol myr^{-1}; $F_{sw} = 9.2 \times 10^{18}$ mol myr^{-1}; $F_{sub} = 4.1 \times 10^{18}$ mol myr^{-1}. The temperature is scaled such that $\Delta T = T - 15.6$ °C. For $\sigma = 0.5$, 0.3 and 0.1, respectively, $F_r = 2.1$, 1.3 and 0.4×10^{18} mol myr^{-1}. These values for F_r are within the lower end of the range estimated by ref. 13, and are reasonable in view of the fact that a steady-state atmosphere/ocean system requires $F_r < F_{sw}$. The system uses a fourth-order Runge–Kutta scheme[20] with time steps of 1 Myr; here time is scaled such that the step increase in β occurs at $t = 0$.

was below the shelf.) Wilkinson and Walker attributed this shift to ecological changes, as have others, but also made a strong case for the alternative of linking β directly to sea level as a result of changes in the area of shallow epicontinental seas. Because β could decrease as sea level rises and vice versa, falling sea level during the past 100 Myr could explain an increase in β. But regardless of the cause, such an increase in β over the past 100 Myr is widely accepted[9–11]. According to Fig. 2, shifts in β of this magnitude should now be affecting the Earth's carbonate–silicate cycle, atmospheric CO_2 and climate.

The value of the subduction parameter σ is highly uncertain. Within our model, the peak of 11 °C in the time history of ΔT with $\Delta B = 0.5$ and $\sigma = 0.5$ is moderated to ~4 °C for $\sigma = 0.7$ and to ~1 °C for $\sigma = 0.9$. Thus substantial forcing towards warming over this 100–200-Myr period is indicated if σ is less than about 0.7. An estimate[13] for σ of between about 0 and 0.5 thus supports such a forcing, but the steady-state assumption used for the present crust–mantle exchange must be treated with caution in view of the possibility of transients as demonstrated here. There is evidence for CO_2 degassing in areas of magmatic and metamorphic activity associated with plate boundaries[2]. Metamorphism of carbonates on subducting slabs is probably the principal source of such outgassing (R. Berner, personal communication). More study of the geochemistry of subducting slabs and the outgassing of CO_2 is required, but it appears quite likely that our present carbon cycle is in a transient resulting from an increase in β.

In reality, cooling has occurred during the past 100 Myr (ref. 16). A decrease in the rate of sea-floor generation and an increase in weathering from the increase in continental area have caused a lower degree of degassing, resulting in a forcing towards cooling of 5–12 °C (refs 2, 3, 5). This could have been balanced by a roughly concurrent forcing-towards-warming, apparent here as an increase in β. But if another, independent, forcing-toward-cooling of about the same absolute magnitude arose from the evolution of angiosperm-deciduous ecosystems[6], the result could have been the net cooling observed. Shifts in continental positions are an additional contributory factor to the observed cooling[17]. In any case, it is likely that the persistence in the recent past of substantial deep-ocean carbonate burial, considered as an isolated effect, is a warming factor today. Given the close relationship in the glacial/interglacial cycle between climate and CO_2 (ref. 18), the substantial deposits of carbonates on the subducting slabs may be contributing to atmospheric CO_2 sufficiently to avoid continuous glacial conditions on the Earth.

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Implosive earthquakes at the active accretionary plate boundary in northern Iceland

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Non-double-couple earthquake focal mechanisms imply a mode of seismic failure other than simple shear, and movements perpendicular to the fault plane. Reports of such events are rare and it is controversial whether such failure can occur at depth in the Earth. Following reports of such earthquakes from the Hengill and Reykjanes volcanic systems on the accretionary plate boundary in Iceland[1–3], a seismological survey was conducted in the Krafla system, Iceland, to explore whether such earthquakes occur elsewhere along the boundary. Here we report the observation of a mixed suite of focal mechanisms at Krafla, including shear events, explosive tensile-crack events such as were observed at Hengill and Reykjanes, and implosive events. These last represent cavity collapse and constitute an entirely new class of earthquake. The Krafla system had undergone a crustal spreading episode[4–10] during the decade before the observations were made, and our results indicate that immediately after such an episode the accretionary plate boundary is characterized by a heterogeneous stress regime, with shear, extensional and compressional sources in close juxtaposition.

In 1985, the earthquake activity associated with the Krafla volcanic system, on the accretionary plate boundary in Iceland, was investigated with a 30-station, temporary seismometer network (Fig. 1). The impetus for this work came from recent, similar experiments in other parts of the spreading plate boundary in Iceland, where small earthquakes with non-double-couple focal mechanisms were discovered[1–3]. In the Hengill area (inset, Fig. 1), about half of the 178 events studied were of tensile-crack type, and at Reykjanes a few similar events were observed. One of the goals of the Krafla experiment was to test the hypothesis that such events characterize the spreading plate boundary in general.

Very accurate hypocentral locations were obtained for 100 small earthquakes ($-2.0 < M_l < 2.1$, where M_l is the coda duration magnitude, normalized to the surface wave magnitude M_s) in the depth range 1–4 km. 25 focal mechanisms were well constrained by P-wave first motions, of which 17 did not violate a double-couple, shear interpretation, three were non-double-couple, explosive, and fitted a tensile-crack interpretation similar to the Hengill and Reykjanes events[1–3], and five were non-double-couple and predominantly implosive. These latter events represent cavity collapse at depths of up to 3.5 km in the Earth's crust. This study provides further evidence for non-double-couple earthquakes. It also indicates an inhomogeneous stress field—in contrast to those from other volcanic systems on the accretionary plate boundary in Iceland, where relatively homogeneous stress fields were revealed[1–3]—and shows compressional phenomena occurring within a regional extensional environment.

The Krafla volcanic system is a segment of the neovolcanic zone in Iceland[11] (Fig. 1). It displays a fissure swarm and a central volcano with a caldera 10 × 7 km in size, underlain by a molten body in the depth range 3–7 km (ref. 4). Two separate geothermal areas lie within the system (Fig. 1). In 1975 the central volcano became active and triggered a spreading episode. For several years its magma chamber inflated continually at a rate of ~5 $m^3 s^{-1}$, and deflated rapidly at intervals of a few months[5–10]. Magma then escaped from the chamber and was intruded along the fissure swarm to form vertical dykes. Up to 8 m of spreading occurred, after which further intrusions could not be accommodated by the altered stress field, and surface magma eruptions occurred[10].

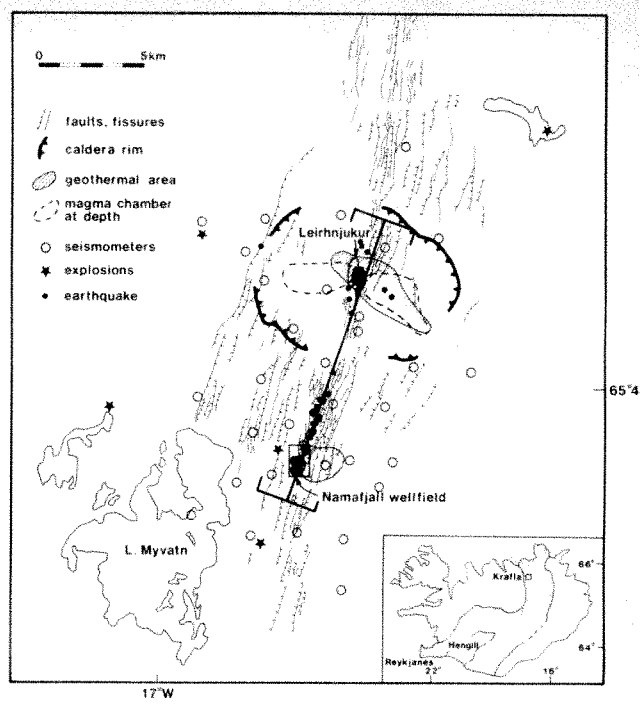


Fig. 1 Tectonic map of the Krafla area, the seismograph network and earthquake epicentres. The outlines of the magma chambers are taken from ref. 4. The bold line corresponds to the section of Fig. 2. Inset shows a map of Iceland. The neovolcanic zone, which marks the locus of the accretionary plate boundary, is shaded, and the locations of the Krafla, Hengill and Reykjanes areas are shown.

At the time of this experiment, the area was volcanically quiescent, and our aim was to study the continuous seismicity associated with the geothermal areas. The majority of earthquakes were located within these areas (Fig. 1) and activity was continuous on a daily basis. It extended to 4 km depth beneath the Namafjall geothermal area in the south, and 3 km depth beneath the Krafla geothermal area in the north (Fig. 2).

The majority of events beneath the Krafla area directly overlie the intersection of the molten body with the 2-km-wide central part of the fissure swarm where most of the spreading occurred[10]. The events form a zone dipping northerly at an angle of 45°, the surface projection of which lies close to the southern caldera fault (Fig. 2), where minor geothermal activity occurs.

Seismic activity within the Namafjall geothermal area clustered in a narrow 'chimney' 2–4 km deep, directly beneath a producing wellfield (Fig. 2). We conclude that these seismic events are induced by heat mining, and the seismically active volume indicates the extent of the cooling zone[2,3,12,13]. Of 20 well constrained focal mechanisms derived for this volume, 16 were predominantly of strike-slip double-couple type with variable orientations of the P and T axes (Fig. 3), and two were predominantly compressive and of tensile-crack type, similar to those observed at Hengill[2,3] and Reykjanes[1] (I and II, Fig. 4*a*). The crack orientation was NNW, oblique to the orientation of the rift zone. In addition, two focal mechanisms displayed

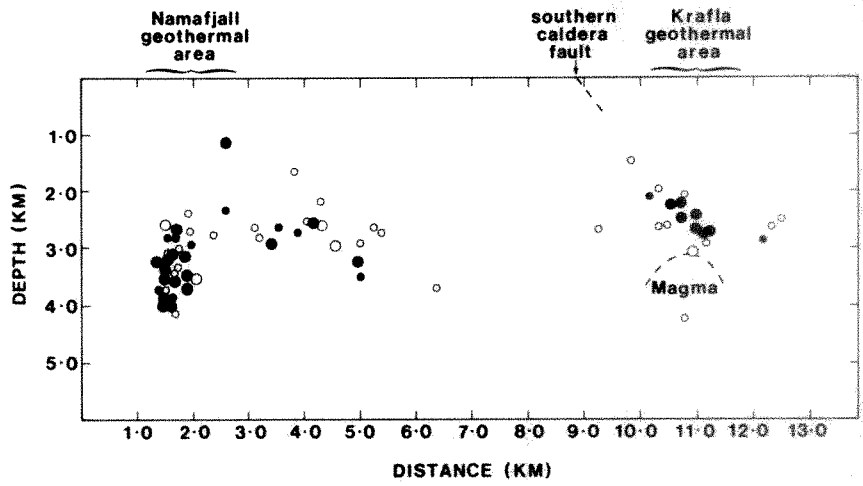


Fig. 2 SSW–NNE cross-section of the region of Fig. 1, showing hypocentral locations.

predominantly dilatational arrivals, and were therefore implosive and indicative of cavity collapse (III and IV, Fig. 4*a*). These results indicate that the seismic volume is deforming chaotically and that the stress field is inhomogenous, in contrast to the Hengill and Reykjanes volcanic systems, where all focal mechanisms were consistent with a uniform extensional stress field.

A third group of events occupy a NNE-trending zone at depths of 2–3.5 km, the locus of recent southerly dyke injections[7]. The four well constrained focal mechanisms obtained for this zone are all non-double-couple, one being of tensile-crack type (III, Fig. 4*b*) and three displaying cavity collapse (I, II and IV, Fig. 4*b*).

Reports of non-double-couple earthquake radiation patterns are rare, so that these data must be examined critically. The P waves were impulsive and did not have the emergent character sometimes observed in volcanic regions, and the seismometer polarities were tested using both explosions and teleseisms. Thus the polarities of the data points presented in Fig. 4 are reliable. Distortion of the ray paths by crustal heterogeneity was ruled out by the refraction-shot data, which showed that the crust is laterally homogeneous to within 5% of the average one-dimensional crustal model used for locating the hypocentres. In addition, the non-double-couple events occurred in the same volume as double-couple events. These factors rule out all explanations, other than non-double-couple source processes, for our observations.

In this spreading environment it is to be expected that the collapsing cavities are cracks orientated parallel to the fissure swarm. The expected radiation pattern of such an event would be omni-dilatational[14]. All events show one or more compressions, however, and in Fig. 4 we show suggested positions for small circular nodal lines that do not violate the data. The seismic moment density tensors of the dilatational events range from $[-1, -1, 0.7]$ to $[-1, -1, 0.3]$. Such sources imply cavity collapse along the edges of the cracks. The event shown in Fig. 4*b*(II) could be fitted only by small circular nodal lines, with the compressive field forming the continuous belt, and such a source represents crack closure. By analogy with source mechanisms that have been proposed to explain the tensile-crack radiation patterns[2,3], the compressive component might be explained by an increase in pore pressure in the crack at the instant of collapse. Such a mechanism would generate small circular nodal lines. An alternative explanation for the compressive field is that it may be generated by a shear component of movement in the source mechanism, in which case the nodal lines would not be small circles.

The non-double-couple events do not exceed magnitudes of $M_L = 0.7$, whereas the double-couple events range in magnitude up to $M_L = 2.1$. The very close correlation of the earthquakes with surface heat loss, and analogies drawn with the Hengill area, are convincing evidence that these events are induced by thermal cracking[2,3,12,13]. The total volumetric component of the earthquakes may be calculated, and compared with the volume contraction resulting from heat loss[2,3]. It is found that the seismic volumetric component is two orders of magnitude smaller than the cooling volumetric component. This result indicates that the majority of the volume contraction resulting from the surface heat loss is accommodated aseismically, a conclusion also reached for the Hengill geothermal area.

The volumetric contraction resulting from surface heat loss reduces the confining stress in the geothermal heat source. In many geothermal areas, this results in continuous, small-magnitude earthquake activity that releases stress in accordance with the regional field[2,3,15–18]. It is to be expected that at the accretionary plate boundary this stress field is uniform and extensional, and that tensile-crack opening occurs[2,3]. This is observed at Hengill[2,3] and Reykjanes[1]. The heterogeneous suite observed at Krafla contrasts with this, probably because of the recent spreading episode, which released the extensional stress field. No such rifting or volcanism is reported for the Hengill and Reykjanes areas for 200 and 700 yr respectively[19,20].

The observations from the Krafla volcanic system provide further evidence that small-magnitude non-double-couple seismic failure characterizes the accretionary plate boundary in

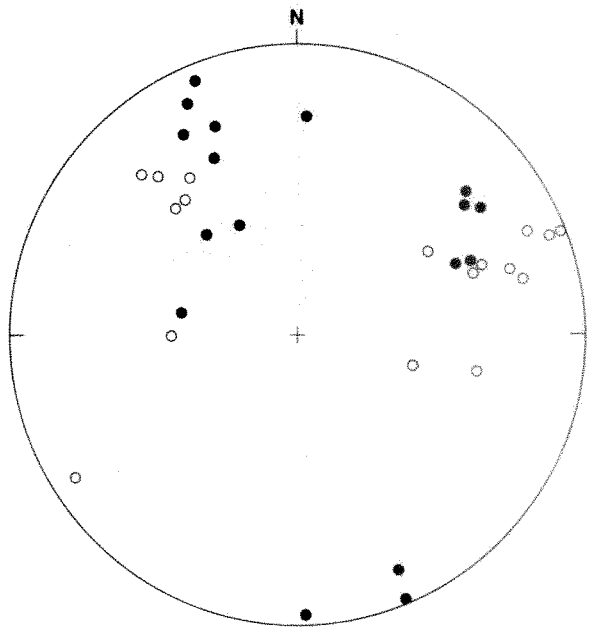


Fig. 3 Stereographic plot of the pressure (○) and tension (●) axes of the double-couple events. These are related to (though not equivalent to) the axes of greatest and least principal stress respectively.

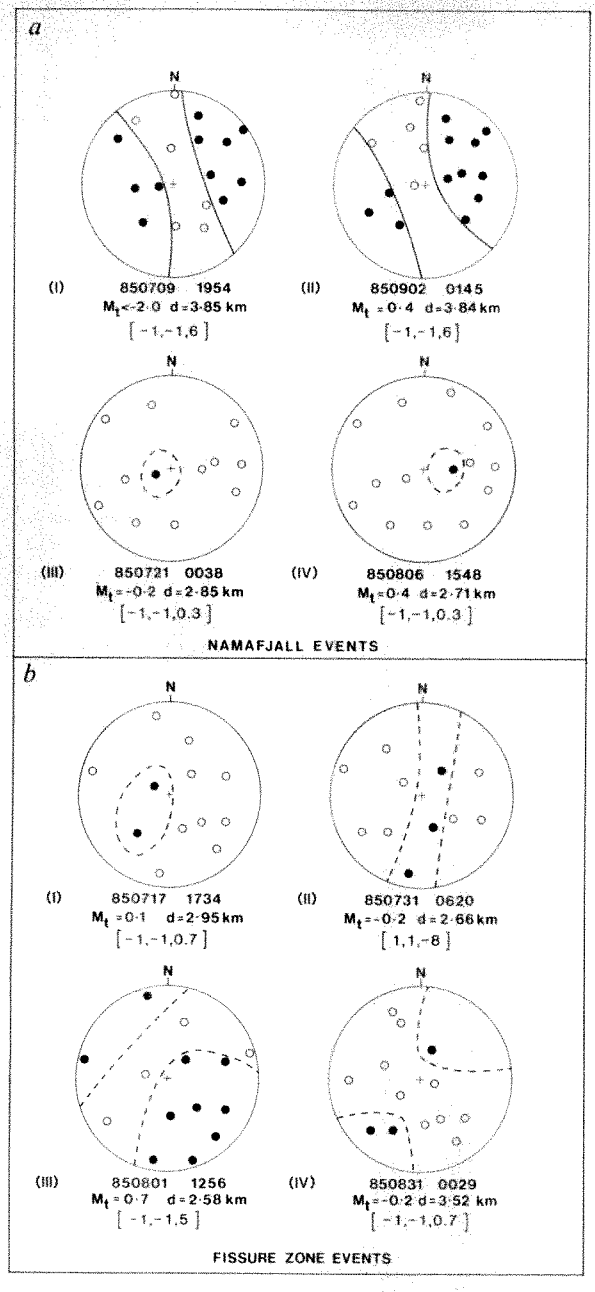


Fig. 4 Non-double-couple focal mechanisms obtained for events beneath the Namafjall geothermal area and along the fissure zone. Filled circles indicate compressions and open circles indicate dilatations. These are upper focal-sphere plots in stereographic projection. Dashed lines are suggested positions of small circular nodal lines. Event captions indicate origin time, local magnitude (M_L), depth (d) and approximate seismic moment density tensor.

Iceland. The Hengill and Reykjanes observations show that an extensional-stress regime may develop during long periods of quiescence between spreading episodes, and that both double-couple and non-double-couple extensional seismic failure occurs. In contrast, the Krafla observations indicate that the immediate post-spreading phase in the accretionary tectonic cycle is characterized by a chaotic stress field, with double-couple events of variable orientation, and both extensional and compressional non-double-couple sources in close juxtaposition.

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Latest Proterozoic plankton from the Amadeus Basin in central Australia

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The plankton of the Proterozoic is preserved mainly as microfossils known as acritarchs, most of which are considered to be cysts of eukaryotic algae. In recent studies of the diversity of Proterozoic and early Palaeozoic acritarchs, a gradual increase in diversity from the Middle into the Late Proterozoic has been shown to be followed by a sharp decrease in diversity in the latest Proterozoic (Ediacarian or Vendian) and then a rise again in the Early Cambrian[1,2]. These observations have been interpreted in terms of the evolutionary diversification of eukaryotic algae and the ecological effects of the pre-Ediacarian glaciation. Occasional discoveries of complex acritarchs in Ediacarian rocks[3-6] have raised some doubts about these interpretations, but more recent work seems to have supported this view[7]. Here we report the discovery of a diverse assemblage of large and morphologically complex acritarchs from the Ediacarian age upper Pertatataka Formation in the Amadeus Basin of central Australia. This, with a recent report of similar fossils from the upper Sinian of China[8], provides a significant new perspective on the history of the plankton. It is now necessary to suggest an earlier radiation, at least in off-shore environments, or to question the reality of the postulated decrease in diversity. In addition, there may well have been an extinction event, late in the Ediacarian. Neither of these phenomena has been recognized previously.

The Amadeus Basin is intracratonic (Fig. 1) and has a sediment fill ranging in age from about 800-900 Myr BP to late Palaeozoic. Dykes in the basement are unconformably overlain by the basal sandstone of the basin and are dated (Rb/Sr on separated minerals) at 897 ± 9 Myr BP providing a maximum age for the sedimentary sequence[9]. The Pertatataka Formation overlies the upper of two Proterozoic glacial sequences and underlies the Arumbera Sandstone which in its lower part contains fossils of the soft-bodied Ediacara fauna (Fig. 2). The upper Arumbera Sandstone contains abundant and diverse trace

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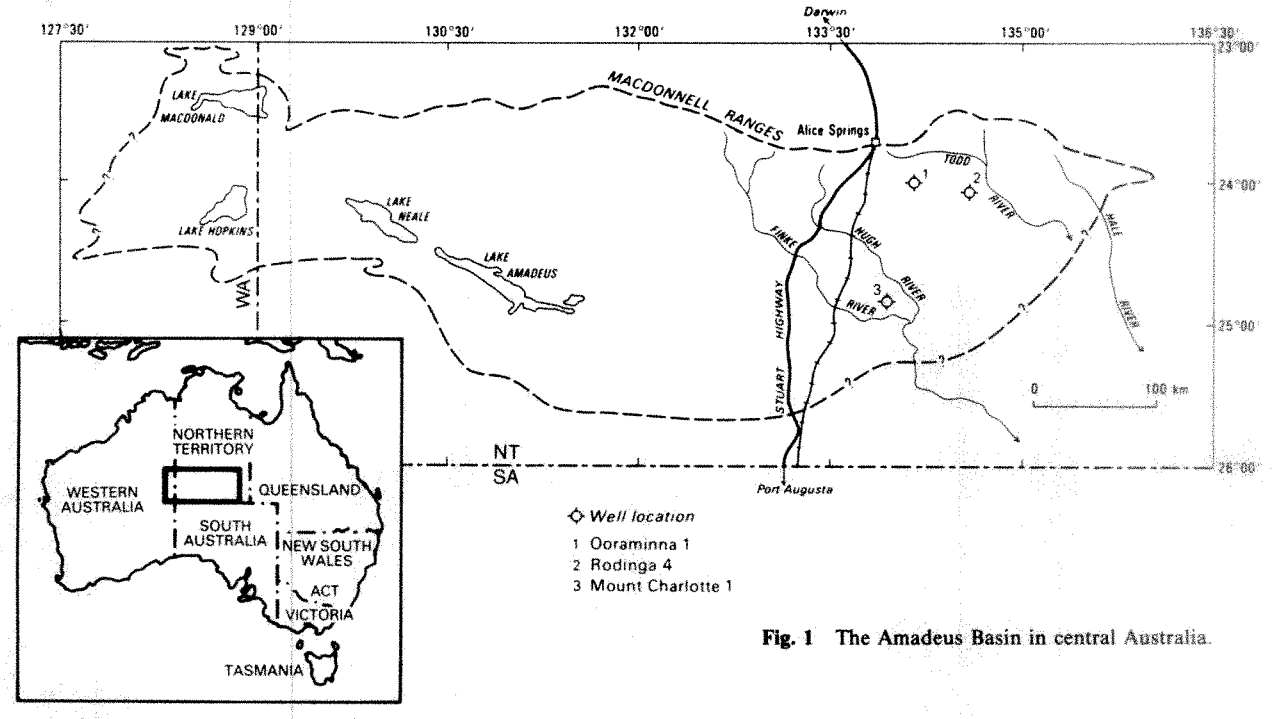


Fig. 1 The Amadeus Basin in central Australia.

fossils like those in Early Cambrian rocks world-wide[10,11], and it in turn is overlain by a carbonate with small shelly fossils of Atdabanian (mid Early Cambrian) or slightly greater age[12]. We can be confident that the Pertatataka Formation is Late Proterozoic, and lithostratigraphic correlations with the nearby Adelaide Geosyncline establish an Ediacarian age[13,14].

The Pertatataka Formation is poorly exposed and for this and other reasons has been little studied, but recent work shows it to be a sequence of distal turbidites. It is made up primarily of red and green shales and siltstones, with minor, thin sandstone beds. The lack of conglomerate and coarse-grained channel sands, the preponderance of base cut-out sequences and the fact that the coarsest grain size is fine sand, are considered to indicate that the turbidites were deposited in an outer submarine fan to basin plain environment[15]. The material used in this study came from oil exploration and stratigraphic drill-holes Ooraminna number 1, Rodinga number 4 and Mt Charlotte number 1 (Fig. 1). We have studied about 40 samples from a stratigraphic interval of 140 m in Rodinga number 4, six samples from Ooraminna number 1 and five samples from Mt Charlotte number 1. The diverse assemblage is restricted to the upper part of the Pertatataka Formation, which occurs in only the first two of these drill holes.

The lower Pertatataka Formation contains what could until now have been regarded as a typical Ediacarian assemblage of acritarchs, consisting of 13 taxa of simple spheroidal forms (the number of taxa must be considered tentative until the taxonomic systematics of this assemblage has been published). The assemblage is not known to include any spinose acritarchs. The spheroidal forms include common species of *Trachysphaeridium*, *Kildinosphaera*, *Protoleiosphaeridium*, *Stictosphaeridium* and *Leiosphaeridia*, as well as some new species. Examples of *Kildinosphaera* are particularly abundant. One sample has very abundant examples of acritarchs comparable to *Bavlinella*, with growth sequences like those described for *Sphaerocongregus*[16].

Possibly as many as 40 species are recognizable in the upper Pertatataka assemblage (again, the number must be treated with caution at this stage). Many of the simple spheroidal forms found in the lower assemblage are also present here, and there are two distinctive new spheroidal forms. One has 12–20-μm cells inside an 80–200-μm-wide vesicle. A second has numerous cells within 150-μm-wide vesicles. Some well preserved filamentous microfossils have been found in samples from Rodinga number 4. Most are fragmentary and were probably transported from a nearshore environment. They are referable to well known taxa such as *Eomycetopsis*, *Siphonophycus*, *Oscillatoriopsis* and the helically coiled *Obruchevella*.

Of most interest are the large and lavishly ornamented forms (Fig. 3). These are abundant and contain more than 50% of all taxa recognizable in the younger assemblage. Most abundant is a form we identify as a new species of *Cymatiosphaeroides*, an acritarch with numerous thin spines between the main vesicle and an outer membrane. This form is about 180 μm wide. There are also large acritarchs (about 180 μm wide) with abundant hair-like spines up to 40 μm long, which we regard as a new species of *Comasphaeridium*. Next most abundant in the assem-

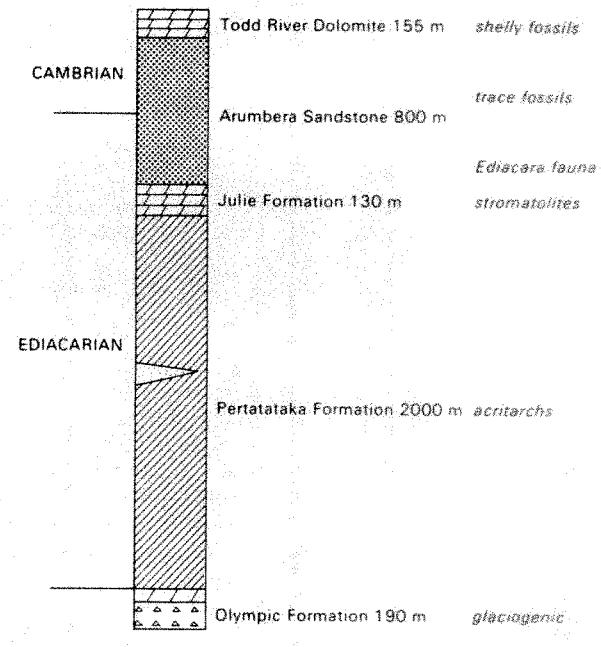


Fig. 2 Generalized stratigraphic section for the NE Amadeus Basin.

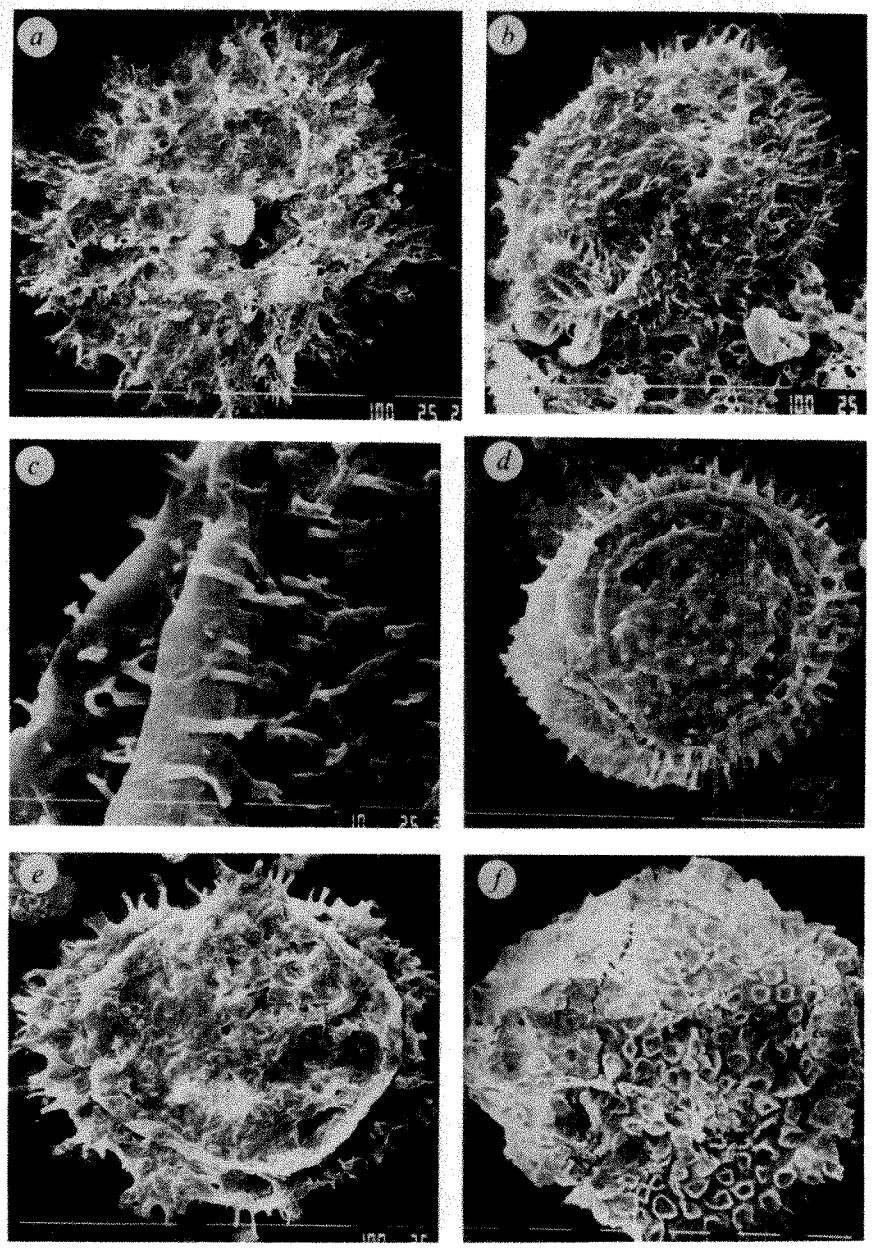


Fig. 3 Acritarchs from the Ediacarian Pertatataka Formation from the indicated depths in BMR drill hole Rodinga number 4 in the Amadeus Basin, central Australia. *a*, Large vesicle with frequently branched processes (34.50–34.76 m). *b*, *Trachyhystrichosphaera* (34–35 m). *c*, *Skiagia* (48.38–48.67 m). *d*, *Gorgonisphaeridium* (53.86–54.14 m). *e*, Unnamed form (52.16–52.50 m). *f*, Unnamed form bearing tube-like processes opening at the outer end but not communicating with interior of the vesicle (48.92–49.24 m). Scale bars, 100 μm for *a*, *b*, *d* and *e*, and 10 μm for *c* and *f*.

blage are very large vesicles with hollow spines; we tentatively identify these as new species of *Baltisphaeridium* and *Trachyhystrichosphaera*. There appear to be several different species present; one form has vesicles up to 240 μm in width and abundant broad-based spines up to 15 μm long. Another has vesicles about 100 μm wide with numerous slender, curved spines up to 40 μm long. Also present is a previously undescribed type of acritarch, with vesicles about 200 μm wide and with two kinds of spines, each confined to discrete areas of the vesicles: one kind is thick and isolated like those of *Baltisphaeridium*, the other thin and hair-like as in *Comasphaeridium*.

Other spinose acritarchs are much less common. Perhaps the most distinctive is a previously undescribed very large acritarch with a vesicle 400 μm wide and numerous thick frequently branched processes up to 60 μm long. Amongst the other spinose forms are some in which the spines have thickened tips, another with broad-based spines like those of *Comasphaeridium*, and a third with blunt-tipped spines. There is also a form with short, branched spines possibly referable to *Gorgonisphaeridium*. Acritarchs up to more than 300 μm wide with short, funnel-shaped processes that open into the vesicles are present in low abundance. We treat these as a new, very large species of the genus *Skiagia*. A rare and completely new form has vesicles 100 μm wide with short, broad, pipe-like processes (Fig. 3*f*).

Several of these genera have previously only been recognized in Cambrian and younger deposits[17–20], although the forms we have discovered are very much larger than their Palaeozoic equivalents. This assemblage is distinctive and different at least at the specific level from younger examples. It is a matter of taxonomic judgement as to whether to include these newly discovered very large acritarchs in genera previously defined for much smaller forms; we have chosen to do so because in all respects other than size our specimens fit the generic diagnoses. Some of the Pertatataka acritarchs also resemble large spinose forms such as *Cymatiosphaeroides* and *Trachyhystrichosphaera* that are rare components of older Late Proterozoic assemblages[21].

Many examples of Ediacarian acritarch assemblages have been described and almost without exception they consist of unornamented spheroidal forms (leiosphaerids) often accom-

panied by macroscopic algae (vendotaenids). A recently described example is that of the Arcoona Quartzite Member, a correlative on the Stuart Shelf of South Australia of the middle Pertatataka Formation of central Australia[7]. However, the new discoveries in the Doushantou Formation of the Yangtse Platform[6,8] and the upper Pertatataka Formation indicate that there was an assemblage of diverse and complex plankters at this time. We consider that there are at least two possible reasons why this assemblage has not been recognized previously: (1) it was not as readily preserved as older and younger assemblages; (2) it lived in an off-shore marine environment that is poorly represented in the Ediacarian geological record. Evidence for the first explanation is the preservation of the assemblage in cherts of the Doushantou Formation[6] but not in interbedded shales. Cherts are well known to be an exceptionally effective medium for the preservation of microfossils, but acritarchs are usually found in shales and siltstones as well as in cherts. Second, few turbidites of definite Ediacarian age seem to have been searched for acritarchs. 'Proterozoic sequences which have been micropalaeontologically investigated so far usually represent shallow marine depositional conditions'[1]. The environment of deposition of the Doushantou Formation is poorly known; pseudomorphs of gypsum are reported from dolomite in the lower part of the formation and ooids from the upper part, suggesting shallow water to emergent environments. The acritarchs come from cherts in flat-laminated silty dolomites in the middle part of the formation, which has been interpreted as having been deposited in a quiet, relatively deep marine environment[22]. The occurrences in the Pertatataka Formation might be the result of unique preservational circumstances, but they certainly suggest that more attention to turbidites is justified.

Previous authors have postulated a sharp decrease in the diversity of the plankton at the time of the widespread pre-Ediacarian glaciation, with no subsequent diversification until Early Cambrian times. It is now necessary to suggest an earlier radiation, at least in off-shore environments, or to question the reality of the postulated decrease in diversity. In addition, there may well have been an extinction event, late in the Ediacarian: the Ediacarian assemblage is very distinctive and different from all younger assemblages, even though we have chosen to place some of the new species in Palaeozoic genera. Many individual Ediacarian acritarchs are some two orders of magnitude greater in volume than comparable forms of Cambrian age. The Proterozoic history of the plankton is still very poorly known and caution is obviously necessary, but our observations do seem to indicate a previously unrecognized extinction event.

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Evolution of seed dormancy

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Palaeozoic fossil conifer cones bearing mature seeds with well developed cotyledonary embryos have been recovered from Permo-Carboniferous strata of North America. These are the most ancient embryos ever discovered within gymnosperm seeds[1,2]. In contrast to the pteridophytic ancestors of seed plants and other major groups of Palaeozoic gymnosperms[3,4], these embryos indicate that there was a significant delay between fertilization and seed germination in the earliest conifers. We suspect that this post-zygotic quiescence may be a first step in the evolution of seed dormancy.

Although gymnosperm ovules from Palaeozoic sediments sometimes contain well preserved gametophyte tissue[5,6], embryos are exceedingly rare[1,2]. Among the hypotheses attempting to account for the paucity of embryos are two that propose that the post-zygotic development in the earliest gymnosperms occurred as a rapid and continuous sequence[3,4]. Modern, and presumably ancient, pteridophytic reproduction is characterized by continuous development from spore germination through to fertilization, embryogeny and sporeling growth[7,8], but the embryos of most modern seed plants have a quiescent period before seed germination and seedling establishment. Moreover, many seeds remain dormant until a physical or physiological barrier has been overcome[9,10]. Dormancy is more common in flowering plants than in gymnosperms, but some degree of quiescence characterizes living seed plants in virtually all the main climatic zones[11–14]. Both mechanisms coordinate seed germination and seedling establishment with favourable environmental conditions.

This report of well developed embryos in Palaeozoic conifer seeds may provide evidence for the evolution of quiescence. The seeds are preserved within the cone of a walchian conifer (Fig. 1*a*) from limestone deposits of either the uppermost Pennsylvanian or lowermost Permian, near Hamilton, Kansas[15,16]. Mature ovules and seeds are 5.5–7 mm long with a well developed integument (Figs 1*a* and 2) and an open micropyle (Fig. 2). The nucellus is thin and contains a large megaspore membrane. Distally the nucellus forms a prominent pollen chamber with a differentiated apical beak (Fig. 2).

Among the 34 seeds present in the Hamilton cone there are cellular megagametophytes in six, and well developed embryos (Figs 1*b* and 2) in four. These numbers are consistent with the low percentage of mature seeds which typically develop in normal cones of modern conifers[17,18]. The fossil seeds show no evidence of larval or fungal damage, and their range of structural variation is consistent with normal developmental variation among healthy seeds of gymnosperms[19,20]. In the Hamilton seeds, cellular gametophyte tissue is preserved in the centre of the megaspore interior. It is unzoned and forms an elongated ellipse with an irregular periphery.

The most mature embryo (Figs 1*b* and 2) is about 2.5 mm long and slender. There are about six cotyledons surrounding the epicotyl, and they are tightly appressed to one another (Figs 1 *b* and 2). The radicle lies adjacent to the pollen chamber, with the cotyledons extending towards the chalaza (Fig. 2). Tracheid maturation is incomplete, but there is a slender zone differentiated at the centre of the embryo (Fig. 1*b*) that may represent procambium. As in modern conifer seeds, the embryo is separated from the gametophyte tissue by a narrow, well defined corrosion cavity (Fig. 1*b*). Large cells at the tip of the radicle seem to be similar to the suspensor tissue of extant conifer seeds[19].

These are the most ancient seeds known to contain cotyledonary embryos, and they are evidence that the embryogeny

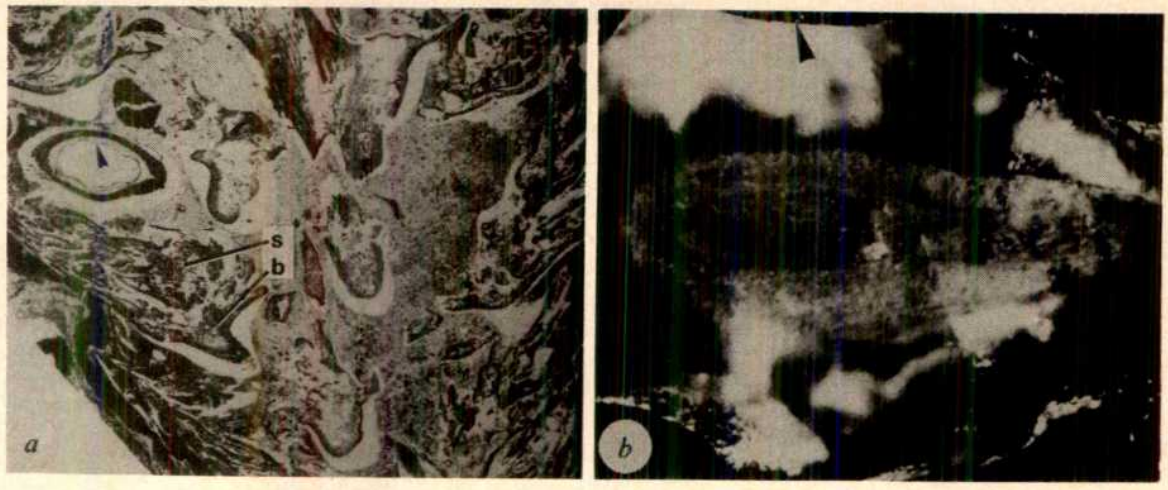


Fig. 1 Walchian conifer cone, bearing seeds with cotyledonary embryos. *a*, Nonmedian longitudinal section of cone showing bracts, fertile shoots and seed (*B*, bract; *S*, shoot) (cone M1613, BL number 32×5); *b*, Reflected light view into the seed cavity of seed in *a*. Embryo with cotyledons at left and radicle at right within cellular megagametophyte tissue (×55). Arrows in *a* and *b* indicate comparable positions on megaspore membrane.

and the bipolar growth of modern gymnosperms characterized primitive Palaeozoic conifers. They also indicate that quiescence before seed germination may have initiated the evolution of seed dormancy. Perhaps more significantly, these seeds provide insight into the selection pressures and developmental mechanisms that could have been associated with the evolution of seed dormancy. Like those of pteridophytes, embryos of the most primitive gymnosperms (including conifer ancestors) presumably had continuous development from the zygote to seedling establishment[3,4]. In the relatively moist lowlands inhabited by the most ancient gymnosperms[21] environmental conditions were probably conducive to seedling establishment most of the time, and immediate germination of seeds probably did not pose a significant reduction in fitness. Immediate germination also would dramatically reduce the probability of seeds with well developed embryos becoming fossilized. But in environments which are more arid or only periodically moist, unregulated germination would lead to the death of a large percentage of seedlings. Under such conditions, delay of seed germination until the onset of the appropriate environmental conditions could impart a significant selective advantage.

As we have suggested here, conifers may have been the most ancient seed plants to display quiescence before seed germination, a possible adaptation for coordinating seed germination with intermittent wetness. We envisage that quiescence, together with specialized features of the vegetative organs, may have dramatically increased physiological tolerance to water stress. The first occurrence of quiescence in these ancient conifers is concordant with the initial establishment of extensive plant communities on relatively dry extra-basinal slopes[23,24] and provides evidence that the origin of seed dormancy contributed to a phenotypic breakthrough[24,25] that facilitated colonization of previously unforested habitats. This emphasizes the role of evolutionary ecology in significantly increasing the numbers and diversity of Palaeozoic conifers as major drying trends produced widespread aridity in the Permian.

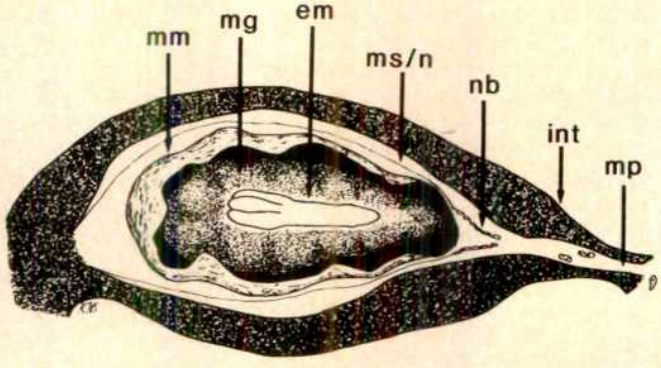


Fig. 2 Reconstruction of seed with embryo in Fig. 1. Based on cone M1613, BL number 17–32. Note pollen in micropyle (*em*, embryo; *int*, integument; *mg*, megagametophyte; *mm* megaspore membrane; *mp*, micropyle; *ms/n*, megasporangium/nucellus; *nb*, nucellar beak).

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Benign familial neonatal convulsions linked to genetic markers on chromosome 20

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Recurrent seizures, commonly known as epilepsies, occur in 1.7% of the general population by age 40[1]. The factors that initiate or underlie seizures are not well understood, but trauma, infectious disease and genetics have been implicated. An understanding of the molecular basis of seizures would shed light on the basic mechanisms of neuronal homeostasis and allow new therapeutic strategies to be explored. Here, we report the mapping of an epilepsy gene to a specific chromosomal region, on the basis of cosegregation of two closely-linked DNA markers with a form of epilepsy known as benign familial neonatal convulsions (BFNC[2], #12120 in ref. 3). The linked markers confirm the genetic basis and autosomal dominant inheritance of this trait, and localize the gene causing BFNC in this family to the long arm of chromosome 20. This regional placement is the first step towards the isolation of a gene involved in neuronal activity in the human brain.

The segregation pattern of BFNC has indicated an autosomal dominant mode of inheritance with high penetrance. Including the original description of this type of seizure by Rett and Teubel in 1964[4], 24 separate pedigrees have been identified[5–13]. Diagnosis of BFNC is based on inheritance, the exclusion of other causes for neonatal seizures, and the developmental pattern of the seizures, which typically start within the first week of life and end spontaneously by six months of age. Subsequent neurological and intellectual development is normal, although a minority (about 10%) of individuals have seizures later in life[5].

We undertook a genetic linkage study of a large, four-generation family (Fig. 1) that included 19 individuals who had documented neonatal or infantile seizures and had met the specific diagnostic criteria for BFNC[14]. Because accurate phenotypic characterization of family members is critical for the success of this approach, the results of laboratory tests for other possible causes of neonatal convulsions were examined for recent generations; all were negative[14]. Pyridoxine dependency was ruled out as the cause of seizures in this family because of the dominant mode of inheritance, the ability of standard anticonvulsants to control the seizures and the absence of mental retardation.

Linkage analysis was performed with the program LINKAGE[15]. We assumed autosomal dominant inheritance of a BFNC gene with a penetrance of 0.9. This conservative assumption of high but incomplete penetrance is supported by published accounts of four families in which an unaffected person has an affected child and also an affected sibling or parent, and by the estimated segregation ratio of 42% in 208 siblings and offspring of probands in 17 BFNC families[8,10,16,17]. Two polymorphic DNA marker loci, CMM6 (D20S19, ref. 18) and RMR6 (D20S20, ref. 19), were found to be tightly linked to the disease locus, giving maximum lod scores (log likelihood ratios) of 3.12 ($\theta = 0.003$) for RMR6 and 2.87 ($\theta = 0.039$) for CMM6; the score for RMR6 corresponds to a likelihood ratio of >1,000/1 in favour of linkage (Table 1).

Joint analysis of the inheritance pattern of the three loci greatly increased our confidence that the two markers are linked to the BFNC gene. In our panel of 59 reference families[20], CMM6 and RMR6 had a maximum lod score at a recombination frequency of 1.6%; when a recombination fraction of 0.016 was assumed for the interval between the two markers in joint analysis with the BFNC locus, the likelihood ratio supporting linkage of the BFNC gene to the two marker loci increased to >500,000/1 (lod score of 5.64, Table 1). The total number of markers tested was 96, and therefore significance for linkage would require a maximum lod score of a least 5.0 (ref. 21). As there are no obligate recombinants in this family we were unable to order the disease locus with respect to the two markers. Because recombinants might be found in a larger sample,

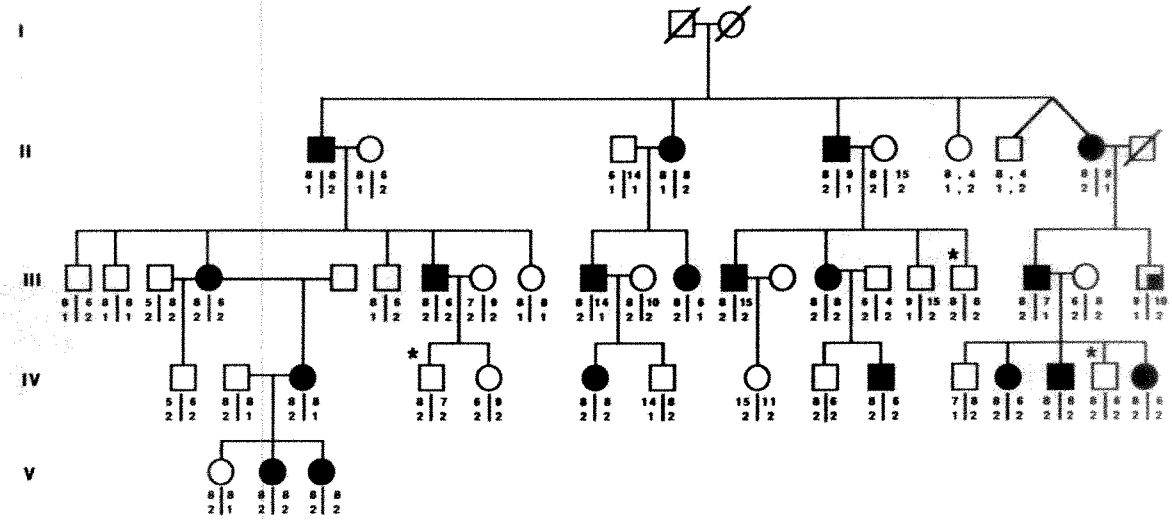


Fig. 1 BFNC pedigree; individuals diagnosed as having the characteristic pattern of seizures are represented by filled circles (females) or squares (males). An individual in generation III with febrile seizures, represented by a filled lower right quadrant, was considered phenotypically unknown for purposes of linkage analysis because his clinical pattern was not typical of BFNC. Under each genotyped individual, alleles are listed for the markers CMM6 (upper) and RMR6 (lower). For the marker locus defined by the probe pCMM6, the restriction enzyme *Taq*I revealed 10 distinct alleles within this family, ranging in size from 2.3 to 6.0 kilobases (kb)[18]. The same enzyme revealed a two-allele polymorphism with the probe pRMR6, with fragment sizes of 2.3 and 2.6 kb[19]. The alleles from the two markers were haplotyped in all except the two individuals in generation II whose allele numbers are separated by commas rather than by a vertical line. Asterisks denote members predicted to possess the mutant gene on the basis of inherited marker alleles, but whose medical records do not indicate the BFNC phenotype.

Table 1 Lod scores (multipoint and pairwise) between BFNC and three DNA markers in a five-generation pedigree

Marker locus	Lod score at recombination fraction of					
	0.00	0.05	0.10	0.20	0.30	0.40
CMM6-RMR6	5.64	5.50	5.20	4.20	2.90	1.40
RMR6	3.12	3.05	2.88	2.32	1.57	0.69
CMM6	2.87	2.92	2.83	2.36	1.63	0.76
MS1-27	−9.11	−3.51	−2.33	−1.12	−0.49	−0.15

Lod scores have been calculated with penetrance = 0.9. When penetrance was varied from 0.70–0.95, maximum lod scores ranged from 5.98–5.02.

however, a confidence interval with boundaries defined by a tenfold decrease in likelihood (the 1-lod confidence interval[22]) must be considered. This boundary for the BFNC gene is found at a recombination frequency of 15% on either side of the two tightly linked markers.

The two DNA marker loci were mapped to chromosome 20 by linkage in reference families with a third DNA marker locus, MS1-27 (D20S4, ref. 23). The MS1-27 locus has been physically mapped to human chromosome 20 using somatic cell hybrids[24], and *in situ* hybridization has confirmed that chromosomal assignment and provided independent, subregional localization of the MS1-27 locus to 20q13.2 (ref. 25). The CMM6 locus gave a lod score of 4.41 with the MS1-27 locus at a recombination frequency of 32.6%, establishing linkage.

We conclude that a mutation at a single genetic locus, located on the long arm of chromosome 20, strongly predisposes individuals in this family to benign neonatal convulsions. Although no obligate recombinants are evident in Fig. 1, the three unaffected individuals indicated by asterisks would be inferred in the linkage analysis to carry the mutation. Without additional information, we cannot determine whether these three subjects reflect recombination events that have occurred between the seizure locus and the marker loci, or reduced penetrance of the BFNC allele.

The next step will be to identify candidate genes from the region of chromosome 20 that seems to harbour the BFNC mutation. Genes that could be considered probable candidates on the basis of their expected physiological role can now be critically tested by determining whether they map to the same region as BFNC; those that map elsewhere can be excluded. For example, a potential candidate for the BFNC mutation might have been the gene encoding glutamate decarboxylase, an enzyme that converts glutamate to the neurotransmitter inhibitor gamma aminobutyric acid; but the glutamate decarboxylase gene is known to reside on chromosome 2[26].

Our observation of genetic linkage not only defines a location, but also confirms the single-gene origin of BFNC in one family. There are few clinical grounds for suspecting that different mutant genes are responsible for BFNC in other families, but they do include an impression that the development of non-febrile seizures after six months of age may be restricted to certain pedigrees. The issue of genetic heterogeneity can be resolved by testing additional families with the two markers described here. Attention then can be directed towards a search for the gene and an explanation for the distinctive developmental pattern of seizures in the BFNC syndrome.

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Arginine vasopressin enhances pH_i regulation in the presence of HCO_3^- by stimulating three acid-base transport systems

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Growth factors raise intracellular pH (pH_i) by stimulating Na^+/H^+ exchange in the absence of HCO_3^- (refs 1–4). In mutant cells that lack the Na^+/H^+ exchange activity, this alkalinization does not occur, and the cells do not proliferate without artificial elevation of pH_i[5,6]. It has therefore been widely suggested that an early pH_i increase is a necessary signal for mitogenesis. In the presence of HCO_3^- however, growth factors fail to raise pH_i in A431 cells[7], renal mesangial cells[8] and 3T3 fibroblasts[9]. In mesangial cells, arginine vasopressin (AVP) raises pH_i in the absence of HCO_3^-, but lowers it when HCO_3^- is present; growth is stimulated under both conditions[8]. We report here that, in the presence of HCO_3^-, AVP stimulates two potent HCO_3^- transporters, as well as the Na^+/H^+ exchanger. These are the Na^+-dependent and Na^+-independent Cl^-/HCO_3^- exchangers. Our results indicate that AVP causes acidification in the presence of HCO_3^- because, at the resting pH_i, it stimulates Na^+-independent Cl^-/HCO_3^- exchange (which lowers pH_i) more than it stimulates the sum of Na^+/H^+ exchange and Na^+-dependent Cl^-/HCO_3^- exchange (both of which raise pH_i). The stimulation of three acid-base transporters by the growth factor AVP greatly enhances the ability of the cell to regulate pH_i.

We examined the effect of AVP in the presence of HCO_3^- on the two acid-extruding transporters previously identified in mesangial cells[10,11]: the Na^+/H^+ exchanger and the Na^+-dependent Cl^-/HCO_3^- exchanger. Figure 1*a* illustrates the effect of AVP on the ability of cells to recover from an acute intracellular acid load imposed by an NH_4^+/NH_3 prepulse[12]. The application

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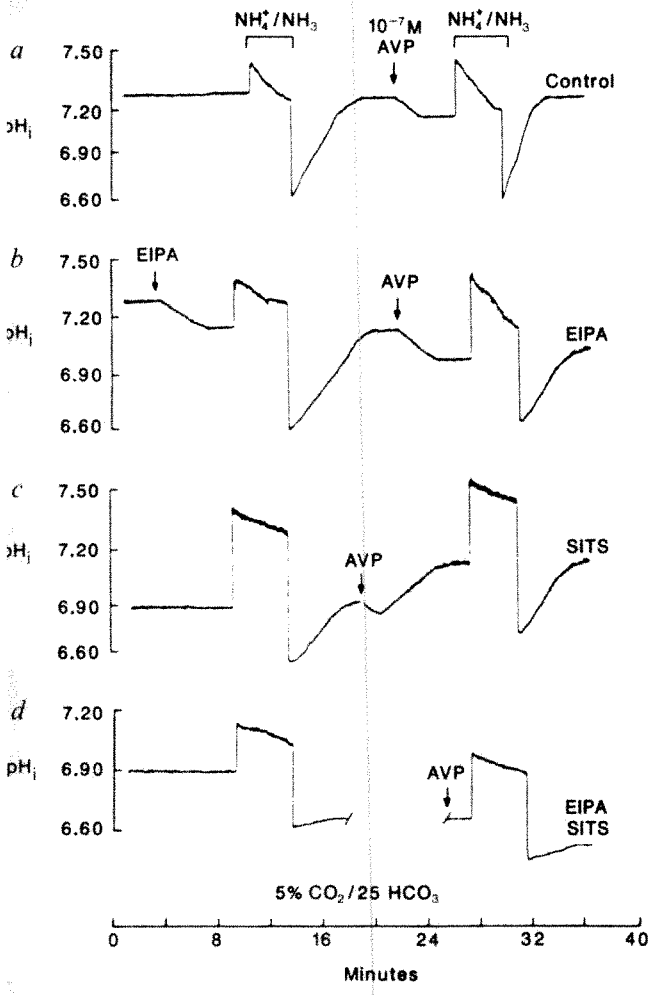


Fig. 1 Effect of 10^{-7} M AVP on the recovery of pH_i from an NH_4^+-induced acid load. For each of the four protocols, the cells were acid-loaded twice by applying and then withdrawing a solution containing 20 mM NH_4^+ (standard HCO_3^- solution (see below) with 20 mM NH_4^+ replacing 20 mM Na^+), first in the absence and then in the presence of AVP. We verified that, in the absence of AVP, the pH_i recovery rates from two consecutive NH_4^+ pulses were within 7±6% (NS) of each other (data not shown). *a*, Control in absence of EIPA and SITS ($n = 14$). *b*, With EIPA ($n = 14$). EIPA (50 μM) was added at the time indicated by the arrow, and was present thereafter. *c*, With SITS ($n = 20$). Because the fluorescence of SITS interferes with that of BCECF (see Methods), SITS was applied to the mesangial cells for 1 hour before the experiment and then washed off vigorously. Addition of AVP to the SITS-pretreated cells produces the pH_i response normally seen in the absence of HCO_3^-, a transient acidification followed by a sustained alkalinization[8]. Thus, the sustained acidification normally produced by AVP in HCO_3^- (*a*) requires a SITS-sensitive transporter. *d*, EIPA plus SITS ($n = 14$). Cells were preincubated for 1 hour in SITS and then washed as in (*c*). EIPA (50 μM) was applied during dye loading and was present thereafter. After EIPA/SITS treatment, the acid extrusion is so slow that pH_i fails to recover beyond ~6.7, and AVP failed to stimulate this slow pH_i recovery.

Methods. Mesangial cells were isolated from glomeruli of young male rats, characterized and maintained in culture as previously described[15]. MCs (passage 2-5) were grown on glass cover slips in mesangial cell growth medium consisting of DMEM plus 20% FCS, 5 μg ml^{-1} insulin, 10 mM L-glutamine, 400 ng ml^{-1} penicillin, 500 ng ml^{-1} streptomycin and 25 mM HCO_3^-. Mesangial cells were rendered quiescent by reducing FCS to 0.5% for 48 h before the pH_i experiments. They were then dye-loaded[8,16] with 10 μM of the acetoxy methylester of BCECF (2,7 biscarboxylethyl-5 (6)-carboxyfluorescein) from a 10 mM stock in DMSO for 20 min at 37 °C. The cover slip with cells was placed in a thermostatically controlled cuvette (37 °C) in a Perkin-Elmer LS-5B spectrofluorometer. We alternately excited the dye at 440 and 500 nm, and measured the fluorescence emission at 530 nm. The BCECF fluorescence excitation ratio was calibrated using the high-K^+/nigericin technique[8,17]. All experiments were performed in CO_2/HCO_3^- solutions. The standard HCO_3^- solution contained (in mM): Na^+, 145; K^+, 5.0; Ca^{2+}, 1.8; Mg^{2+}, 1.0; Cl^-, 122; HCO_3^-, 25; SO_4^{2-}, 1.0; phosphate, 1.0; and glucose, 10, and was equilibrated with 5% CO_2/95% O_2 at pH 7.40. EIPA was a gift of Dr Burckhard (Frankfurt, FRG).

and subsequent removal of 20 mM NH_4^+ causes pH_i to decrease rapidly. The intracellular pH recovers from this acid load because of the activity of the ethyl isopropylamiloride (EIPA)-sensitive Na^+/H^+ exchanger, which accounts for about one-third of acid extrusion[10], and the SITS-sensitive Na^+-dependent Cl^-/HCO_3^- exchanger, which accounts for about two-thirds[11] (see Table 1 legend). After application of 10^{-7} M AVP, pH_i falls slowly as previously described[8], and recovery from an NH_4^+-induced acid load is substantially faster. In 14 experiments, AVP increased the mean rate of pH_i recovery ($d(pH_i)/dt$), at $pH_i = 6.60$, from 77.6 to 145.2×10^{-4} pH units $second^{-1}$ ($P < 0.001$). The total buffering power (β_{total}) was 22.5 and 23.7 mM per pH unit in the presence and absence of AVP respectively. Thus, the net acid-extrusion rate ($J_{ext} = d(pH_i)/dt \times \beta_{total}$) increased from 183 to 326 μM s^{-1} (see Table 1).

To determine the proportion of the increased J_{ext} that is due to stimulation of Na^+/H^+ exchange, we repeated the experiment in Fig. 1*a*, but blocked Na^+/H^+ exchange with 50 μM EIPA (Fig. 1*b*). Application of EIPA causes a sustained pH_i decrease, indicating that Na^+/H^+ exchange is active in the presence of HCO_3^-. EIPA also slows the pH_i recovery from NH_4^+-induced acid loads, both in the absence and presence of AVP. Table 1 includes a comparison of results of experiments such as those in Fig. 1*a* and *b*. In the absence of AVP, EIPA reduces J_{ext} from 183 to 121 μM s^{-1}, so that the EIPA-sensitive component of J_{ext} (that is, Na^+/H^+ exchange) is 62 μM s^{-1}. In the presence of AVP, EIPA reduces J_{ext} from 326 to 205 μM s^{-1}, so that the EIPA-sensitive component of J_{ext} is 121 μM s^{-1}. Thus, AVP increases Na^+/H^+ exchange activity from 62 to 121 μM s^{-1}. Nevertheless, this increment (59 μM s^{-1}) accounts for less than half the total increase in J_{ext} (142 μM s^{-1}) elicited by AVP.

We performed the experiment shown in Fig. 1*c* to determine whether stimulation of the Na^+-dependent Cl^-/HCO_3^- exchanger accounts for the rest of the AVP-elicted increase in J_{ext}. Mesangial cells were pre-incubated for 1 hour with 0.5 mM SITS, a treatment that permanently blocks both the Na^+-dependent and Na^+-independent Cl^-/HCO_3^- exchangers[10,11]. The mean initial pH_i (6.94±0.04) after SITS pretreatment was significantly lower ($P < 0.001$; $n = 20$) than for matched controls (7.30±0.03), indicating that the acid-extruding Na^+-dependent Cl^-/HCO_3^- exchanger is more active than the acid-loading Na^+-independent exchanger in the normal steady state. As shown in Fig. 1*c*, the pH_i recovery rate from an acid load is substantially slowed by SITS pretreatment, both in the absence and presence of AVP. The data are summarized in Table 1. In the absence of AVP, SITS reduces J_{ext} from 183 to 62 μM s^{-1}, so that the SITS-sensitive component of J_{ext} (HCO_3^- transport) is 121 μM s^{-1}. In the presence of AVP, SITS reduces J_{ext} from 326 to 90 μM sec^{-1}, so that the SITS-sensitive component of J_{ext} is 236 μM s^{-1}. Thus, AVP nearly doubles HCO_3^--dependent acid extrusion, from 121 to 236 μM s^{-1}. This underestimates the stimulation of Na^+-dependent Cl^-/HCO_3^- exchange, because SITS pretreatment also blocks the Na^+-independent Cl^-/HCO_3^- exchanger.

If the AVP-induced increase in J_{ext} is due to the dual stimulation of Na^+/H^+ exchange and Na^+-dependent Cl^-/HCO_3^- exchange, then the application of both EIPA and SITS would block the effect of AVP. Cells preincubated with SITS and then exposed to EIPA exhibit little recovery from an acid load, with or without AVP (Fig. 1*d*). In six other experiments (data not shown) we blocked Na^+-dependent Cl^-/HCO_3^- exchange by pretreating cells for 1 hour in a medium lacking chloride, and blocked Na^+/H^+ exchange with EIPA. The results were identical to those shown in Fig. 1*d*. Because the SITS-sensitive component of AVP-stimulated acid extrusion is also Cl^- dependent, it is not mediated by the introduction of a new Cl^--independent mechanism, such as Na^+/HCO_3^- cotransport[13].

Because AVP stimulates both acid extruders, it could lower pH_i (see Fig. 1*a*) only if an acid-loading mechanism were

Table 1 Effect of EIPA and SITS on acid extrusion rate J_{ext} after a 20 mM NH_4^+ prepulse J_{ext} ($\mu M\ s^{-1}$)

	Control	EIPA	SITS	EIPA-sensitive (Na^+/H^+ exchange)	SITS-sensitive (HCO_3^- transport)	Acid-loading
−AVP	183 ± 4	121 ± 2	62 ± 2	62	121	0
+AVP	326 ± 12	205 ± 10	90 ± 3	121	236	31
AVP-dep.	142 ± 8			59	115	31
% Stimulation	78			95	95	

Fluxes were calculated as the product of $d(pH_i)/dt$ and β_{total} ($\beta_{total} = \beta_{HCO_3^-} + \beta_{non\text{-}HCO_3^-}$). Values of $d(pH_i)/dt$ were determined at pH_i 6.6 $\beta_{non\text{-}HCO_3^-}$, 13.4 mM per pH unit in the presence and 14.6 mM per pH unit in the absence of AVP ($n = 10$), was measured by briefly exposing mesangial cells to 10 mM NH_4^+ in the absence of Na^+ and HCO_3^-. $\beta_{HCO_3^-}$ was 9.1 mM per pH unit at pH_i 6.6. AVP increases the total J_{ext} by 142 instead of the expected 174 $\mu M\ s^{-1}$ (59 + 115 $\mu M\ s^{-1}$). This implies that AVP not only stimulates the two acid extruders, but also increase EIPA/SITS-insensitive acid loading to 31 $\mu M\ s^{-1}$, whereas in the absence of AVP there is no discrepancy. Thus, the growth factor substantially increases background acid-loading. SITS is 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate, DIDS is 4,4′-diisothiocyanostilbene-2,2′-disulphonate.

stimulated even more. To determine whether AVP enhances Na^+-independent Cl^-/HCO_3^- exchange, we determined the Cl^-/HCO_3^- exchange rate from the pH_i increase induced by acute removal of external Cl^- (ref. 11). As shown in Fig. 2*a*, Cl^- removal reverses the exchanger, producing an HCO_3^- influx that raises pH_i. This alkalinization is DIDS-sensitive (see Table 1 legend), and is larger and faster in the presence of AVP. AVP increased Na^+-independent Cl^-/HCO_3^- exchange by 129%, increasing the DIDS-sensitive J_{ext} from 313 to 717 $\mu M\ s^{-1}$ (Table 2). The Na^+-dependent Cl^-/HCO_3^- exchanger, which is relatively inactive at pH_i values at or above normal, does not contribute to the pH_i increase elicited by Cl^- removal. In the absence of Na^+, AVP increased the DIDS-sensitive alkalinization induced by Cl^- removal by 112% ($n = 14$), close to the stimulation observed in the presence of Na^+ (data not shown).

Table 2 Effect of DIDS on equivalent net influx of HCO_3^- (J_{ext}) induced by Cl^- removal

	J_{ext} ($\mu M\ s^{-1}$)		
	Control	DIDS	DIDS-sensitive (HCO_3^- transport)
−AVP	333 ± 30	20 ± 1	313 ± 20
+AVP	780 ± 40	63 ± 3	717 ± 40
AVP-dep.	447 ± 3	43 ± 4	404 ± 13
% Stimulation	134 ± 14		129 ± 17

Fluxes calculated as in Table 1, but at pH_i 7.5. $\beta_{HCO_3^-}$ was 72.5 mM per pH unit at pH_i 7.5.

An alternative assay for Na^+-independent Cl^-/HCO_3^- exchange is to acutely load the cells with alkali and monitor the rate at which pH_i returns to normal. We alkali-loaded the cells by applying and then withdrawing a solution buffered to pH 7.4 with 10% CO_2/50 mM HCO_3^-. The introduction of this solution caused a sustained pH_i increase (Fig. 2*b*); this alkalinization was absent in cells pretreated with SITS (data not shown). When the mesangial cells were returned to 5% CO_2/25 mM HCO_3^-, pH_i rapidly increased because of CO_2 efflux), representing an acute alkali load. The subsequent recovery to the original pH_i is probably due to Na^+-independent Cl^-/HCO_3^- exchange, in that the equivalent HCO_3^- efflux (J_{load}) is blocked by more than 90% by either DIDS or Cl^- removal (Fig. 2*b* and Table 3). The Na^+-dependent Cl^-/HCO_3^- exchanger does not contribute to this pH_i decline: this transporter is normally an acid extruder, and it is virtually inactive at high pH_i[14]. AVP strongly stimulates the DIDS-sensitive/Cl^--dependent pH_i recovery from an alkali load (Fig. 2*c*). The DIDS-sensitive component of J_{load} (Table 3) is increased from 568 to 1,385 $\mu M\ s^{-1}$, a stimulation of 143%. The DIDS-insensitive and Cl^--independent components of the pH_i recovery are not significantly affected by AVP. Thus, Cl^-/HCO_3^- exchange is stimulated by more than 100% by AVP. Because AVP produces a fall o steady-state pH_i in control conditions, its effect in the physiologi cal pH_i range must be to increase the amount of acid-loadin by Na^+-independent Cl^-/HCO_3^- exchange more than th amount of acid extrusion by the other two transporters.

The growth factor-induced increase in pH_i is not observed i the presence of HCO_3^- despite suggestions that it may be

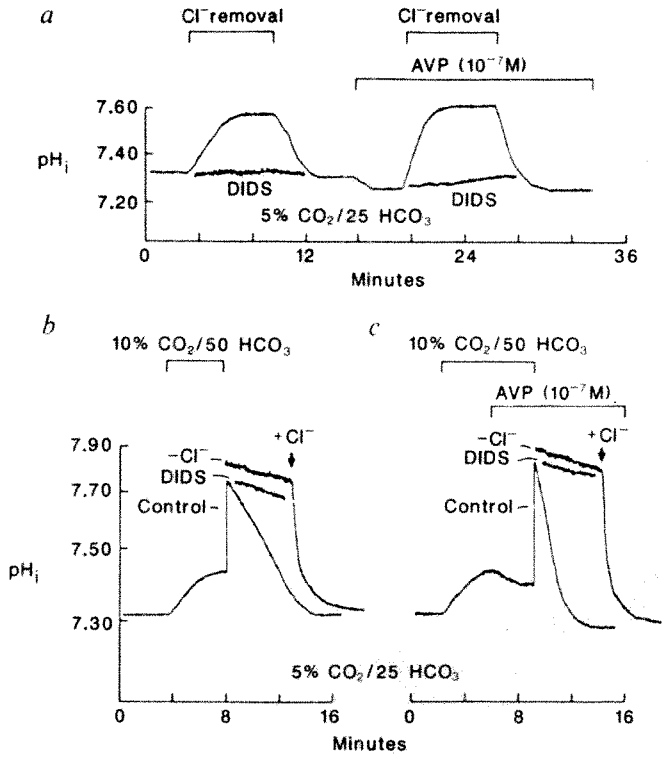


Fig. 2 Effect of 10^{-7} M AVP on Na^+-independent Cl^-/HCO_3^- exchange. *a*, Cl^-/HCO_3^- exchange assessed by Cl^- removal (Cl^- replaced by aspartate) and readdition ($n = 16$). Separate experiments were performed in 50 μM DIDS, which does not interfere with BCECF fluorescence ($n = 16$). *b*, Cl^-/HCO_3^- exchange assessed by pH_i recovery from acute alkali load in the absence of AVP. The alkali load was imposed by applying and withdrawing 10% CO_2/50 mM HCO_3^- (standard HCO_3^- solution, with 25 mM extra HCO_3^- replacing 25 mM Cl^-). The 10% CO_2/50 mM HCO_3^- solution caused pH_i to increase by 0.12 ± 0.03 ($P < 0.01$). The experiment was performed under control conditions ($n = 12$), in the presence of 50 μM DIDS ($n = 12$), and with Cl^- replaced with aspartate ($n = 12$). *c*, Cl^-/HCO_3^- exchange assessed by pH_i recovery from acute alkali load in the presence of AVP. As in (*b*), the experiment was performed under control conditions ($n = 12$), in the presence of 50 μM DIDS ($n = 12$), and in the absence of Cl^- ($n = 12$).

Table 3 Effect of DIDS and chloride absence on acid-loading rate (J_{load}) following a 10% CO_2 prepulse

	J_{load} ($\mu M\ s^{-1}$)			
	Control	DIDS	No chloride	DIDS-sensitive (HCO_3^- transport)
−AVP	630 ± 22	62 ± 3	51 ± 5	568
+AVP	1,504 ± 76	119 ± 9	80 ± 3	1,385
AVP dep.	874 ± 35	57 ± 8	31 ± 7	717
% Stimulation	140 ± 11			143

Fluxes calculated as in Table 1, but at pH 7.7. $\beta_{HCO_3^-}$ was 114.9 mM per pH unit at pH_i 7.7.

crucial mitogenic signal. It could be advantageous for an activated cell to maintain a steady-state pH_i close to the unactivated level given the sensitivity to pH of nearly all cellular processes. Such a steady-state pH_i is achieved when processes that acid-load the cell (for example Na^+-independent Cl^-/HCO_3^- exchange) balance those that alkali-load it (for example, Na^+/H^+) and Na^+-dependent Cl^-/HCO_3^- exchange). It would also be advantageous however, for the activated cell to be able to recover more rapidly from acid and alkali loads. The requirements for both a near-normal steady-state pH_i and enhanced pH_i regulation can only be met if increases in acid loading and acid extrusion by the growth factor are comparable. This is precisely the response of the mesangial cell to AVP: application of the growth factor causes only a small decrease in steady-state pH_i, but enormous increases in the rate at which the cell recovers from both acid (Fig. 1*a*) and alkali loads (Fig. 2*c*). This effect of AVP on pH_i does not seem to be an isolated event, as we have found eight other growth factors that also produce only a small pH_i decrease in the presence of HCO_3^- (data not shown). Thus, an important physiological effect of the growth factor is to maintain a near-normal pH_i while stimulating several acid-base transporters.

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A pentapeptide as minimal antigenic determinant for MHC class I-restricted T lymphocytes

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Peptides that are antigenic for T lymphocytes are ligands for two receptors, the class I or II glycoproteins that are encoded by genes in the major histocompatibility complex, and the idiotypic α/β chain T-cell antigen receptor[1–9]. That a peptide must bind to an MHC molecule to interact with a T-cell antigen receptor is the molecular basis of the MHC restriction of antigen-recognition by T lymphocytes[10,11]. In such a trimolecular interaction the amino-acid sequence of the peptide must specify the contact with both receptors: agretope residues bind to the MHC receptor and epitope residues bind to the T-cell antigen receptor[12,13]. From a compilation of known antigenic peptides, two algorithms have been proposed to predict antigenic sites in proteins. One algorithm uses linear motifs in the sequence[14], whereas the other considers peptide conformation and predicts antigenicity for amphipathic α-helices[15,16]. We report here that a systematic delimitation of an antigenic site precisely identifies a predicted pentapeptide motif as the minimal antigenic determinant presented by a class I MHC molecule and recognized by a cytolytic T lymphocyte clone.

Synthetic peptides have been derived from the amino-acid sequence of pp89, an immediate-early (IE) phase regulatory protein of murine cytomegalovirus[17–20]. The 19-mer P(161–179) contained within its 595 residues[18] is an antigenic sequence[21]. This sequence H_2N-[161]GRLMYDMYPHFMPTNLGPS[179]-COOH (one-letter code) contains the predicted motif HFMPT[14]. By screening a series of related peptides that had been reduced in length from both terminals (not all shown), we found that the nonapeptide YPHFMPTNL represents the optimal antigenic peptide (Fig. 1 and Table 1) for the cytolytic T lymphocyte (CTL) IE1 clone[22,23]. Even though binding of peptides to class I molecules has not yet been demonstrated directly, the selective recognition on L/L^d cells implies that the L^d molecule is the

Table 1 Delimitation of the antigenic motif for CTL clone IE1

	Peptide	Recognition (competition)	Peptide concentration [log M] detection limit	detection saturation
11-mer:	MYPHFMPTNLG	+	−7 to −6	−4 to −3
10-mers:	MYPHFMPTNL	+	−9 to −8	−7 to −6
	YPHFMPTNLG	+	−8 to −7	−6 to −5
9-mers:	MYPHFMPTN	−(−)		
	YPHFMPTNL	+	−12 to −10	−9 to −7
	PHFMPTNLG	+(−)	−4 to −3	−2
8-mers:	YPHFMPTN	−(−)		
	PHFMPTNL	+(−)	−4 to −3	
7-mers:	YPHFMPT	−		
	PHFMPTN	+	−8 to −7	−4
	HFMPTNL	+	−5 to −4	
6-mers:	YPHFMP	−		
	PHFMPTN	+	−2 to −3	
	HFMPTN	+	−2	
	FMPTNL	−		
5-mer:	HFMPT	+	−3	
4-mers:	HFMP	−		
	FMPT	−		

Dose-response titrations of peptides give the peptide molarities in solution required for detectable target formation (detection limit) and for optimal target formation (detection saturation). The concentration ranges are compiled from at least 3 and up to 12 independent experiments.

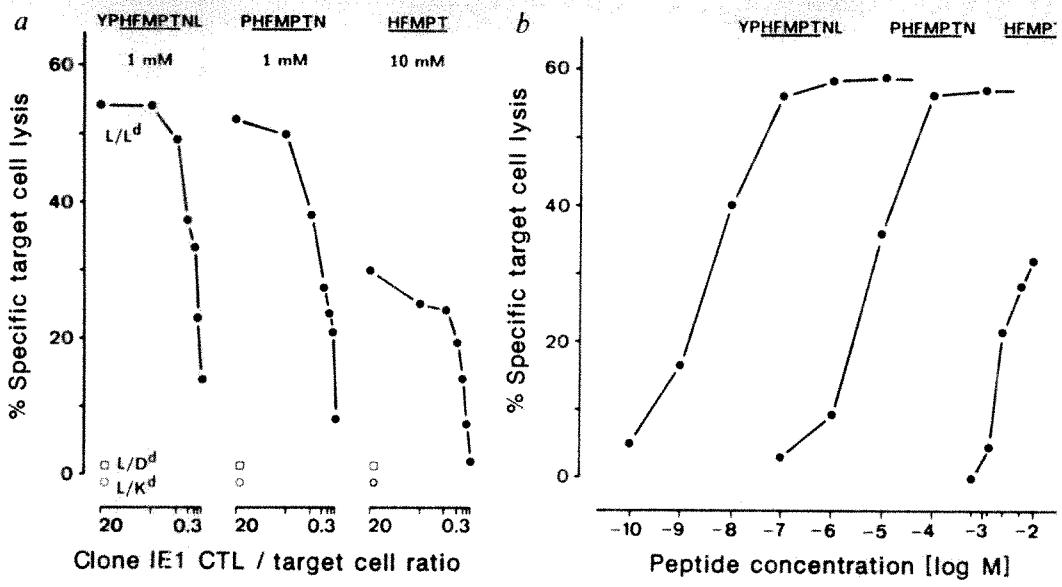


Fig. 1 Cytolytic assays demonstrating L^d-restricted recognition of peptides by CTL clone IE1 (*a*), and dose-response titrations of peptides (*b*). Each value in the peptide titrations represents the lysis determined from the plateau of a complete effector-to-target titration graph at a ratio of 20, and each value of % specific lysis is the mean of four replicate determinations.

Methods. Cytolytic effector cells: The murine (BALB/c strain; MHC^d) CTL line IE1.13-IL was derived from clone IE1 by recloning[22,23]. Clone IE1 expresses an α/β (Vβ6) TCR/CD3 complex and displays the surface phenotype $CD4^-CD5^-CD8^+Thy\text{-}1^+$. L fibroblast transfectants: L/L^d cells were derived from L cells by transfection with the L^d gene, and L/D^d and L/K^d transfectants were provided by M. Cochet and J. P. Abastado (Institut Pasteur). Surface expression of the L^d, D^d and K^d molecules was confirmed by cytofluorography with monoclonal antibodies. Synthetic peptides: Peptides were synthesized by using the Barany-Merrifield solid-phase technique on an Applied Biosystems Peptide Synthesizer, purified preparatively and analysed as described previously[13]. Preparation of target cells and cytolytic assay: Target cells were labelled at 37 °C for 1 h with $Na_2[^{51}Cr]O_4$. The radioactive cells were then distributed in aliquots of 10^5 cells and incubated in 0.2 ml for a further 1 h at 27 °C with peptides at defined molarities (1 M corresponds to 10^{15} peptide molecules per cell) dissolved in RPMI 1640 culture medium supplemented with 2% of FCS. The highest peptide concentration tested was 10^{-2} M. This limit was imposed by the solubility of the peptides. After washing to remove excess peptide and ^{51}Cr, a standard 3-h cytolytic assay was performed at 37 °C with 1,000 target cells and graded numbers of clone IE1 CTL. Competition assays: The ability of peptides to compete with the optimal antigenic nonapeptide YPHFMPTNL was tested in two ways. Either the concentration of the antigenic peptide was kept constant at 10^{-7} M and the competitor peptide titrated up to 10^{-4} M, or the concentration of the competitor peptide was kept constant at 10^{-4} M and the antigenic peptide was titrated from 10^{-5} to 10^{-10} M. In both cases the L/L^d target cells were pre-incubated for 30 min with the competitor peptide before the antigenic peptide was added.

receptor involved in the interaction with the T-cell antigen receptor (TCR) of clone IE1 for the family of peptides tested (Fig. 1*a*). Titration of the nonapeptide YPHFMPTNL, the heptapeptide PHFMPTN, and the pentapeptide HFMPT resulted in dose-response saturation curves showing thousandfold differences in antigenic potency (Fig. 1*b*). The data refer to a one-hour incubation of target cells with peptide. Differences in antigenic potency are also reflected by the time needed for optimal target formation; five minutes is long enough to prepare an optimal nonapeptide target, which is in line with data for a high-affinity peptide[24], whereas plateau lysis could be achieved with the pentapeptide only when the peptide pulse was prolonged to two hours or more (not shown). Because clone IE1 does not discriminate between nonapeptide and pentapeptide target once the target is formed, the limiting step is apparently the association between the peptide ligand and its MHC receptor on the target-cell surface. We therefore conclude that HFMPT forms the antigenic core of the peptides by comprising both the complete epitope for the TCR of clone IE1 and an agretope that is adequate, but not optimal for specification of the interaction with L^d.

The deletion of Tyr1 (Y) from peptide YPHFMPTNL diminished and the deletion of Leu 9 (L) destroyed the antigenicity, and the resulting octapeptides PHFMPTNL and YPHFMPTN both also failed to compete with YPHFMPTNL (Table 1). According to the currently used theorem for classifying residues as T-cell contact residues or as MHC-molecule contact residues[12], Tyr 1 and Leu 9 would have been interpreted as residues contacting the MHC molecule. This conclusion, however, was proven incorrect by the findings that PHFMPTN, which lacks both residues, was $>10^3$-fold more antigenic than PHFMPTNL (Table 1) and could not be competed by a 10^3-fold excess of YPHFMPTN (not shown), although both octapeptides include the heptapeptide sequence entirely. Results compiled in Table 1 follow a consistent pattern from the pentameric cor motif up to the nonapeptide size, in that symmetric addition of residues improved the antigenicity, whereas addition o residues at either end had a negative effect.

This communication adds new aspects to the current understanding of the MHC molecule-peptide-TCR interaction. The first aspect concerns the minimal length of antigenic peptides Until now, the shortest peptides reported as antigenic were heptapeptides presented by MHC class II molecules[25,26]. A recent study described a nonapeptide presented by an MHC class I molecule as minimal antigenic peptide of the glycoprotein of LCMV[27]. It should be emphasized that in our example systematic shortening from both termini was critical for the identification of the pentapeptide as the minimal antigenic peptide. Since the sequence of this pentapeptide is a predicted pentameric motif, and as most motifs are tetrameric[14], recognition even of tetrapeptides may be possible.

The second aspect concerns the classification of epitope and agretope residues. For the hen egg-white lysozyme peptide (52-61), Allen *et al.*[12] defined residues as MHC-antigen contact residues when a substitution led to the loss of both antigenicity and the ability to compete with the unmodified antigenic peptide. With this approach and proposing a helical conformation, agretope residues were segregated from epitope residues. From a set of overlapping peptides, Sette *et al.*[28] predicted a heptameric core in a planar conformation for the ovalbumin peptide (323-339). In contrast to these examples, we have positively identified the motif HFMPT as an antigenic core in pp89 peptides by demonstrating a direct antigenicity of the pentapeptide HFMPT. The important implication is that residues whose deletion causes loss of both antigenic potency and competitive ability are not necessarily agretope residues in the classical sense of MHC-receptor binding sites. We propose that residues flanking an antigenic core motif can affect the antigenic potency positively

or negatively by their influence on peptide conformation.

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Class I cross-restricted T cells reveal low responder allele due to processing of viral antigen

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Cytotoxic T lymphocytes (CTL) recognize protein antigens which have been processed by the target cell and then presented in association with the relevant class I molecule of the major histocompatibility complex (MHC)[1]. Short synthetic peptides, which are able to associate directly with target cells[1], may substitute for these processed fragments in stimulating antigen-specific CTL responses. Using this approach, a dominant HLA-A2-restricted epitope has previously been mapped to residues 58–68 of influenza A virus matrix protein[2]. Here we report HLA-A2-restricted CTL which are also able to recognize this short synthetic peptide in association with HLA-Aw69, but which fail to recognize HLA-Aw69 expressing cells infected with influenza A virus. Furthermore, individuals possessing HLA-Aw69 who respond to influenza A virus, do not respond to M58–68. These results imply that the low response to this epitope on infection of HLA-Aw69 individuals with influenza A is due to failure of the naturally processed product of matrix protein to associate with Aw69.

Individuals who express the common HLA-A2 variant, HLA-A2.1 (HLA-A*0202 as defined in ref. 3), and who can mount a cytotoxic response to influenza infected target cells, following *in vitro* stimulation with virus have been shown, in all cases tested to date, to possess CTL that specifically recognize synthetic peptides representing residues 56–68 of the matrix protein[4]. Individuals who have other variants of A2, or the closely related allele HLA-Aw69, which differs by six amino acids from A2*0202 (listed in Table 1)[5], did not respond to this region of matrix protein[4].

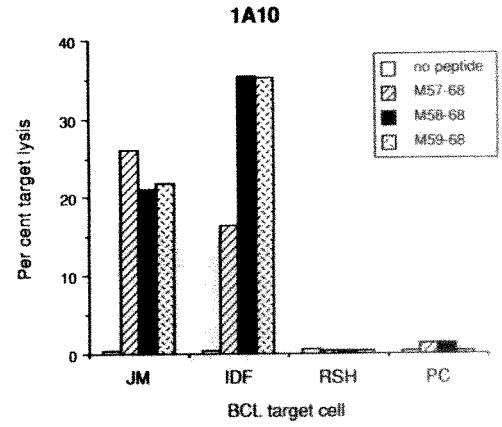


Fig. 1 CTL are cross-restricted, recognizing peptide on both autologous and Aw69-expressing target cells. Recognition by CTL clone 1A10 from the donor JM (A2, 2; B15, 51; DR4, 4) of peptides M57–58, M58–68, and M59–68 of influenza matrix protein on autologous BCL target cells (JM), three HLA-mismatched BCL, IDF (A26, w69; B18, 38; DR11, 11), RSH (A68, 68; B42, 42; DR3, 3) and PC (A3, 24; B37, 62; DR6, 6). $K/T = 1.5$, peptides present throughout assay 10 μM.
Methods. CTL clone 1A10 was derived from a parent line from the donor, JM. Peripheral blood lymphocytes were separated from whole blood, stimulated initially with A/X31 virus (grown and stored as previously described[18]) and maintained in culture by repeated stimulations with peptide M57–68, as previously described[4]. CTL were cloned by selection of CD8 positive cells (labelled with B941 antibody from Dr C. Mawas, and fluorescein-labelled anti-mouse immunoglobulin) using an Ortho Cytofluorograf cell sorter, into 96-well plates containing 10^4 irradiated BCL (pretreated with 50 μM M57–68) and IL-2 10 U ml^{-1} (Cetus Corp.) in RPMI containing 10% fetal calf serum. Monoclonality was indicated by the presence of a single β-chain gene rearrangement on Southern blotting (data not shown). Cytotoxic activity was assessed by incubating EBV-transformed lymphoblastoid cells (BCL), labelled with ^{51}Cr, with CTL and diluted peptide (where appropriate) for 4 h in round-bottomed 96-well plates. Per cent target lysis was calculated from the formula $(E - M/D - M) \times 100$ where E = experimental ^{51}Cr release, M = release in presence of culture medium (always <18%) and D = release by 5% Triton X-100. For details of the peptides see legend to Fig. 3.

We made several CTL lines and clones from a single HLA-A2 expressing individual, JM, some of which were able to recognize matrix peptide 56–68 in association with HLA-Aw69 as well as A2, although they were otherwise HLA-restricted (Fig. 2). CTL clone 1A10 was able to recognize the short synthetic peptides M57–68, M58–68 and M59–68 in association with both the autologous (JM) HLA-A2-expressing EBV-transformed B lymphoblastoid cell line (BCL), and a BCL expressing Aw69 which did not share any HLA alleles with JM (Fig. 1), but did not recognize two other HLA mismatched BCL in the presence of these peptides.

Although these matrix-specific A2-restricted CTL were able to recognize Aw69-expressing target cells in the presence of synthetic peptide, a discrepancy between recognition of A2 and Aw69 BCL was seen following A/X31 virus infection of the target cells (Fig. 2). Both autologous and A2-matched BCL were recognized following pretreatment with the peptide, or infection with influenza virus (Fig. 2*a*, *e*). In contrast Aw69-expressing BCL were very poorly recognized following virus infection, although they were well recognized after peptide treatment (Fig. 2*b*, *c*, *f*). In addition, virus-specific polyclonal CTL from an Aw69-expressing donor were unable to recognize the synthetic peptide M58–68 (Fig. 2*d*). Even with repeated stimulation of these latter CTL with M58–68 *in vitro*, we were unable to obtain a response from either of two Aw69-expressing (HLA identical)

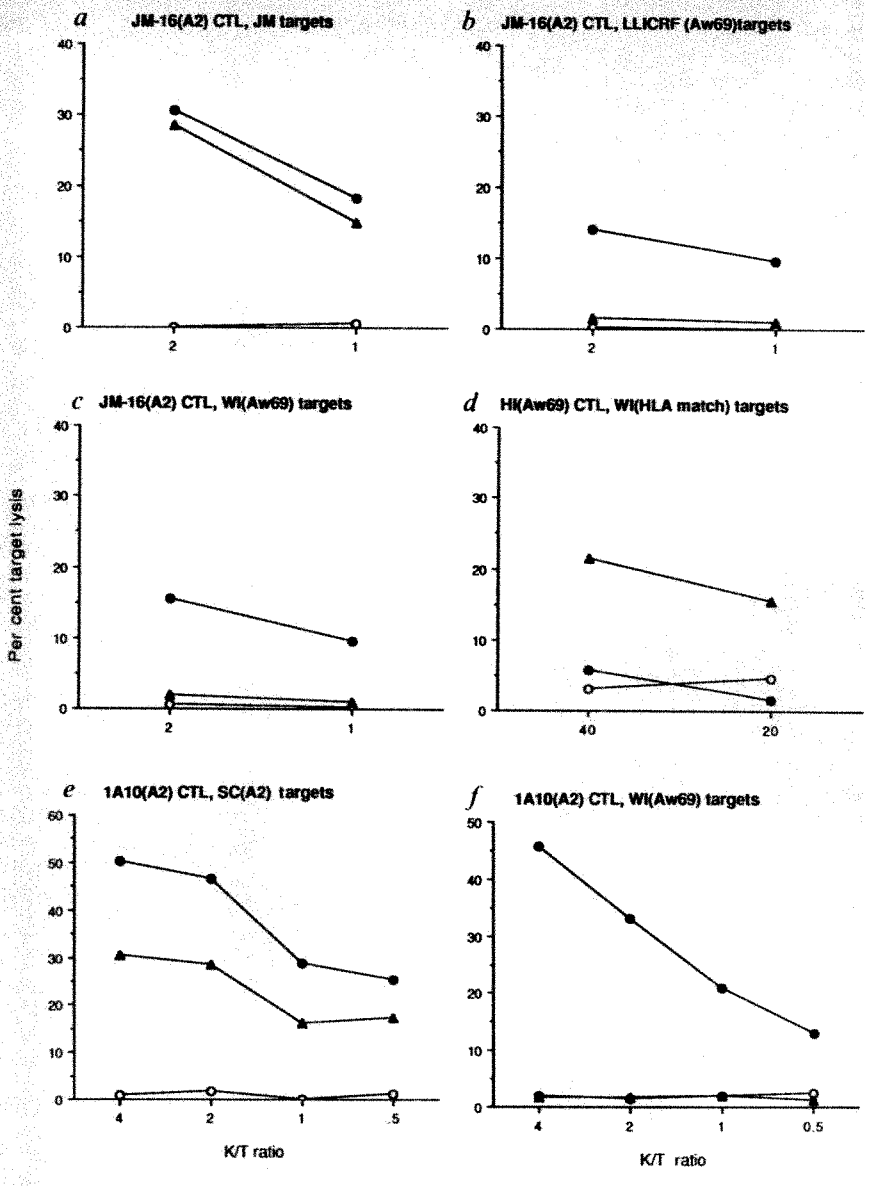


Fig. 2 HLA-Aw69-expressing BCL can present peptide, but not virus to A2-restricted matrix specific CTL, whereas an Aw69-expressing donor is able to respond to virus, but not matrix peptide. Polyclonal CTL line JM-16 was assayed for recognition of autologous BCL (*a*), or Aw69-expressing BCL LLICRF (A1, w69; B8, 35; DR10, 17) (*b*) or WI (Aw68, w69; B22, 51; DR2, 13) (*c*); target cells (10^4 per well), were either untreated (open circles), pretreated with 50 μM M58–68 (closed circles) or infected with A/X31 (closed triangles). Polyclonal CTL from donor HI (HLA-identical brother to WI) were assayed for recognition of WI target cells (*d*). In a separate experiment, the A2-restricted CTL clone, 1A10 (derived as in Fig. 1), was assayed for recognition of a BCL matched only through A2, (SC: A2, 2; B27, 27; DR2, 2) (*e*), or the Aw69-expressing BCL, WI (*f*).

Methods. JM-16 was derived as described in the legend to Fig. 1. Polyclonal CTL from donor HI were prepared by stimulating peripheral blood lymphocytes with A/X31 virus in culture for 7 days, as previously described[4]. The ^{51}Cr-release assay, was performed as for Fig. 1 except that BCL target cells were preincubated for 1 h with either medium, peptide or A/X31 virus, washed, and then rested in 1 ml medium at 37 °C for 3 h before ^{51}Cr-labelling, and addition to the assay.

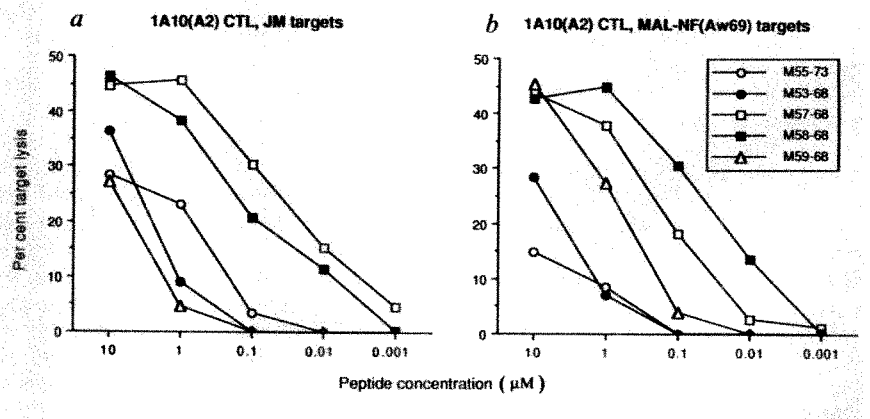


Fig. 3 The pattern of peptide recognition varies depending on whether the target cell expresses A2 or Aw69. CTL clone 1A10 ($K/T = 2$) was assayed for recognition of peptides of varying lengths in association with autologous BCL (*a*) or a BCL expressing Aw69 (MAL-NF: A1, w69; B35, 57; DR10, 17) (*b*) using 10^4 target cells per well. Non-specific lysis, that is in the absence of peptides, was 0% for both targets.

Methods. Influenza A virus matrix peptides M55–73 (leu-thr-lys-gly-ile-leu-gly-phe-val-phe-thr-leu-thr-val-pro-ser-glu-arg-gly); M53–68 (ser-pro-...) M55–68, M57–68, M58–68 and M59–68 were synthesized and donated by Dr J. Rothbard. The ^{51}Cr-release assay was performed as for Fig. 1.

donors. Thus, although Aw69 was able to present the synthetic peptide to CTL, Aw69 donors were non-responders to this epitope.

It is possible that patterns of immunodominance in a T-cell response may be influenced by other MHC molecules[5] or by other background genes[6]. But Aw69 target cells with different MHC types were all poor presenters to the A2-restricted CTL following virus infection (an example is shown in Fig. 2*b*). Therefore we propose that the Aw69 molecule itself, although binding the synthetic peptide well, may associate poorly with a naturally produced fragment. Peptide M58–68 is the optimum length for maximal target lysis by matrix-specific CTL restricted through HLA-A2 and cross-restricted through Aw69. There is, however, no evidence that this is the actual length of peptide produced when whole matrix protein is processed by a cell. We therefore assayed for killing of A2 or Aw69 targets using a

Table 1 Amino-acid differences between the sequences of HLA-A*0202 and HLA-Aw69

Position	A*0202	Aw69	Location†
9	Phe	Tyr	β-sheet, in
62	Gly	Arg	α-helix, up
63	Glu	Asn	α-helix, in
66	Lys	Asn	α-helix, in
70	His	Gln	α-helix, in
74	His	Asp	α-helix, in

† All amino-acid differences are in the α_1 domain, orientation is indicated, either pointing into the purative peptide binding site (in), or pointing towards the T-cell receptor (up), according to the crystal structure determined by Bjorkman *et al*[8,9].

number of peptides of varying lengths from this region (Fig. 3). The dose response curves of recognition by the CTL clone 1A10 of peptide M58–68 were very similar for both A2- (autologous) and Aw69-expressing target cells (Fig. 3). But recognition of the shorter peptide, M59–68 was more efficient on the Aw69 target, whereas recognition of the longer peptides, particularly M55–73, was approximately tenfold less efficient. This decreased efficiency may be sufficient to account for the low response if natural peptides are present in low concentrations.

HLA-Aw69, a rare allele, is the result of a natural exon shuffle between HLA-A2 and HLA-Aw68 (ref. 7) with the α_1 domain from Aw68 and the α_2 and α_3 domains from A2. Although there are only six amino-acid differences between Aw69 and A2 (listed in Table 1), reference to the crystal structure of A2, as defined by Bjorkman *et al*[8,9], suggests that these occur in areas likely to be important for either peptide binding or direct recognition of the MHC molecule by the T-cell receptor. Cross-restriction for recognition of foreign antigen by class I-restricted T cells has been reported infrequently. Human HLA-Aw69 restricted Epstein-Barr virus-specific CTL were able to recognize Aw68 (sharing the α_1 domain), but not A2-expressing BCL[10]. In mice, an exchange of the exons coding for the α_1 and α_2 domains of H-2 L^d and D^d maintained epitopes recognized by CTL specific for influenza or Sendai virus but not vesicular stomatitis virus[11]. Other reports of exon shuffles between the α_1 and α_2 domains have not identified cross-restricted CTL[12–14].

Cross-restricted CTL recognizing cyanogen bromide fragments of ovalbumin on target cells expressing three different H-2 types have been generated by priming *in vitro*[15,16] but these T cells were unable to recognize target cells expressing a transfected ovalbumin gene. A similar result was found with MHC class II-restricted T-cell clones, produced by priming mice with peptide *in vitro* in that the clones responded on processing and presentation of the native molecule[17]. In both these cases, the T-cell responses were generated against peptides that were not epitopes when the animals were primed with the native proteins. Here, we show that HLA-Aw69 can present a synthetic peptide to A2-restricted CTL, induced by natural infection, even though Aw69 is a non-responder allele to the native virus antigen. All of these results imply that the processing system limits the complexity of naturally induced T-cell immune responses.

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Characterization of the yeast *HSP60* gene coding for a mitochondrial assembly factor

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The hsp60 protein isolated from the protozoan *Tetrahymena thermophila* is induced in response to heat stress and is a member of an immunologically conserved family represented in *Escherichia coli* and in mitochondria of plants and animals[1,2]. We report here the cloning and characterization of a nuclear gene, *HSP60*, which codes for the hsp60 homologue from the yeast *Saccharomyces cerevisiae*. Nucleotide sequence analysis revealed that yeast hsp60 is related to the groEL protein of *E. coli*[3–6] and the RUBISCO-binding protein (RBP) of chloroplasts[7]. *HSP60* was found to be the genetic locus of the conditional-lethal mutation described by Cheng *et al.*[8], which at non-permissive temperature is defective in the assembly of several different multisubunit complexes in mitochondria. These data are consistent with the hypothesis that the groEL-related proteins serve an evolutionarily conserved function as accessory factors facilitating the folding and/or association of individual subunits of multimeric protein complexes[7,9–14].

Polyclonal antiserum raised against purified *T. thermophila* hsp60 (ref. 1) was used to isolate a segment of the yeast *HSP60* gene by screening an expression library of genomic DNA fragments ligated into the λ-phage vector λgt11 (ref. 15). Analysis of a 3.0 kilobase (kb) yeast DNA fragment present in one positive clone revealed an open reading frame coding for a protein homologous to both groEL and RBP. We used this DNA as a hybridization probe to isolate an overlapping segment of the yeast genome which contained the entire *HSP60* coding sequence (Fig. 1).

The sequence of a 1.8-kb region containing *HSP60* is presented in Fig. 3, along with the derived amino-acid sequence. The *HSP60* gene codes for a polypeptide of 572 amino acids (relative molecular mass (M_r) 60,830) which is similar to both groEL and RBP (Fig. 4). Based on our previous finding that the hsp60 family of proteins is structurally and immunologically similar to groEL[2], we have defined this open reading frame as the yeast *HSP60* gene. Northern hybridization analyses showed that *HSP60* is transcribed into a mRNA of ~1.9 kb, commensurate with the estimated 64,000 (64K) M_r of hsp60 (Fig. 2). This mRNA is induced 2–3-fold above basal levels when cells are heat-stressed, in agreement with the heat-shock inducibility of hsp60 (refs 1, 2) (Fig. 2). Increasing the copy number of *HSP60* by transformation with the multicopy plasmid YEpHSP60 (Fig. 1) results in higher concentration of immunoreactive material in mitochondria (Fig. 2), providing further evidence for the identity of this gene.

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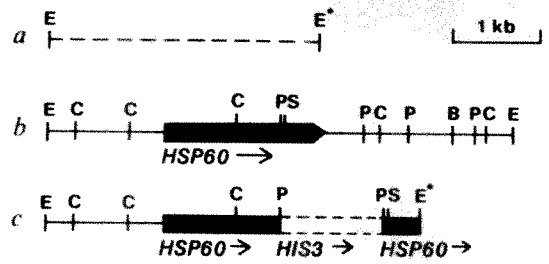


Fig. 1 Restriction maps of *HSP60* and *hsp60::HIS3*. *a*, The dotted line indicates the region of yeast genomic DNA isolated from a λgt11 clone which reacts with polyclonal antiserum raised against hsp60 purified from *Tetrahymena*[1]. The *Eco*RI site designated E* was inserted as a linker during construction of the library. *b*, restriction map of the genomic DNA fragment containing the region depicted in *a*. The black bar indicates the coding region of *HSP60* as determined by nucleotide sequence analysis, with the direction of transcription indicated by the arrow. Plasmid YEpHSP60 is comprised of this 5.3-kb *Eco*RI fragment cloned in the 2μ circle-based shuttle vector YEp352 (ref. 20). *c*, Restriction map of the null allele *hsp60::HIS3*. Black bars indicate the coding regions of *HSP60*, and dotted lines indicate a *HIS3*-containing fragment which interrupts *HSP60* by insertion at the *Pst*I site. The positions of restriction enzyme recognition sites are indicated for *Eco*RI (E), *Bcl*I (C), *Pst*I (P), *Sal*I (S) and *Bam*HI (B).

Methods. A library of yeast genomic DNA fragments in λgt11 (ref. 15) was screened with a polyclonal antiserum reactive with hsp60 (ref. 1). From 300,000 library recombinants screened, a single positive plaque was isolated which upon repurification gave only positive clones. The positive recombinant contained three *Eco*RI fragments within the λ arms, one of which hybridized to a heat-shock-inducible mRNA. This 3.0-kb fragment is depicted in *a*. Nucleotide sequence analysis of the ends of the 3.0-kb fragment revealed an open reading frame homologous to *groEL* which continued through the site marked E* in the 5′–3′ direction. Therefore, we used the 3.0-kb fragment as a hybridization probe to isolate an overlapping genomic fragment containing the entire *HSP60* gene. Southern hybridization analysis showed that the 3.0-kb fragment isolated from λgt11 was derived from a genomic *Eco*RI fragment of ~5 kb. A library of *Eco*RI fragments with a size of ~5 kb was preapred in the 'phagemid' vector pUC118 (ref. 21) and screened with the 3.0-kb fragment. Positive clones contained the 5.3-kb *Eco*RI fragment shown in *b*. Nucleotide sequence analysis (Fig. 3) revealed the location of *HSP60*. To construct *hsp60::HIS3* the 3.0-kb *Eco*RI fragment in *a* was cloned into the plasmid vector YEp352E, a derivative of YEp352 from which the *Pvu*II fragment containing the partial *lac* operon and multiple cloning site was removed and replaced with an *Eco*RI linker. The recombinant plasmid was linearized at the unique *Pst*I site within *HSP60* and ligated to a 1.15-kb fragment of yeast DNA containing the wild-type *HIS3* gene[22]. One of the *Pst*I sites delimiting this fragment is located downstream of the *HIS3* coding region[22], whereas the other *Pst*I site is derived from the pUC18 multiple cloning site. The linear *Eco*RI fragment shown in *c* was excised from YEp352E and used to transform the *his3⁻/his3⁻* diploid a/αW03 to histidine independence[23]. Southern hybridization analysis of chromosomal DNA verified that the His⁺ transformants had integrated the disrupted allele at the homologous site in one chromosome, and were of the genetic constitution *HSP60/hsp60::HIS3* (data not shown).

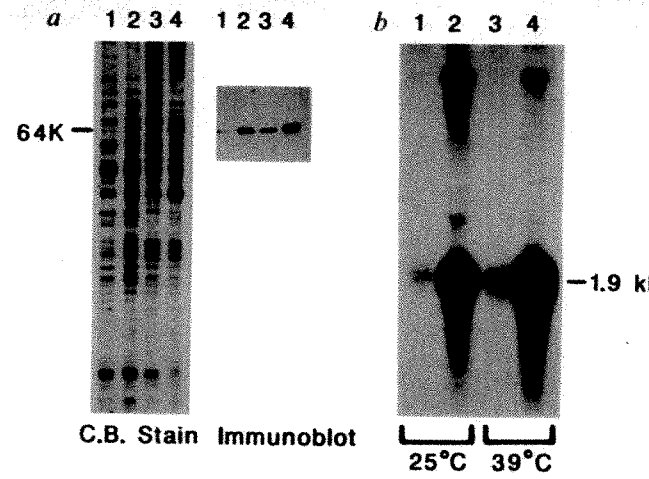


Fig. 2 *a*, Immunoblot analysis of cells containing single or multiple copies of *HSP60*. Haploid strain aW303 (lanes 1, 3) or the same strain transformed with YEpHSP60 (lanes 2, 4) were grown to mid-log phase at 30 °C. Sixty micrograms of total protein extracts[26] (lanes 1, 2) or purified mitochondria[27] (lanes 3, 4) were run in duplicate SDS-polyacrylamide gels. Proteins in one gel were stained with Coomassie blue, whereas those in the second gel were transferred to nitrocellulose paper and probed with hsp60-reactive antiserum as previously described[1]. Raising the copy number of *HSP60* by including the gene on a multi-copy plasmid increases the cellular level of hsp60-specific immunoreactive protein. Relative molecular mass standards included on the stained gel (not shown) showed the immunoreactive material to be of M_r 64K, in agreement with our previous analyses[1,2]. Thus, there is a discrepancy between the apparent M_r of hsp60 determined by polyacrylamide gel electrophoresis and the value predicted by the nucleotide sequence of the gene (61 K including the putative mitochondrial targeting peptide). *b*, Steady state levels of *HSP60* mRNA in normal and heat-stressed conditions. Strain aW303 (lanes 1, 3) or the same strain transformed with YEpHSP60 (lanes 2, 4) were grown to mid-log phase at 25 °C. The cultures were then divided and grown for a further 2 hours either at 25 °C (lanes 1, 2) or 39 °C (lanes 3, 4). Total nucleic acids were extracted[28], denatured with glyoxal[29], and separated by agarose gel electrophoresis. Nucleic acids were transferred to nitrocellulose filters and analysed by Northern blotting, using the 3.0-kb fragment shown in Fig. 1*a* as a radiolabelled probe. The probe hybridizes to a single RNA band of 1.9 kb (RNA relative-molecular mass standards not shown). The concentration of this mRNA is two to three times higher in the heat-shocked cells than in the non-stressed control. Raising the copy number *HSP60* increases the steady-state level of the 1.9-kb mRNA at 25 °C, and heat-shock induction is again observed. The high M_r band seen in lanes 2 and 4 is due to hybridization of the probe to DNA.

Comparison of the *HSP60* and *groEL* products reveals 54 per cent amino-acid identity, given five small insertion/deletions, whereas comparison with RBP reveals 43 per cent identity including four insertion/deletions (Fig. 4). Regions of similarity are distributed evenly throughout the lengths of the proteins; one exception occurs at the carboxyl terminus, where the repeated Gly-Gly-Met motif is conserved in both hsp60 and groEL, but not in RBP. Hsp60 contains an amino-terminal extension of ~22 amino acids which is not represented in two other members of this gene family. This sequence is characteristic of most mitochondrial targeting peptides[16] in that it contains five basic and no acidic residues, and contains a total of six hydroxylated residues. The presence of this targeting sequence is consistent with the mitochondrial location of hsp60 (refs 1, 2).

One-step gene disruption[17] was used to construct an allele of *HSP60* which is inactive because of insertion of the *HIS3* gene (Fig. 1). The diploid strain a/αW303∇HSP60 was constructed with one wild-type allele and one null allele designated *hsp60::HIS3*. This strain is homozygous *his3⁻*, so the wild-type and null alleles of *HSP60* can be scored by histidine auxotrophy and prototrophy, respectively. Following dissection of tetrads derived from a/αW303∇HSP60, only two of the four meiotic products from each ascus formed colonies on non-selective medium; replica plating revealed that all surviving progeny required histidine and thus had received the wild-type allele. Because no haploid cells containing *hsp60::HIS3* form colonies, we conclude that *HSP60* is essential for cell viability in yeast. Cells were grown at 30 °C, thus the requirement for *HSP60* is not limited to conditions of heat stress.

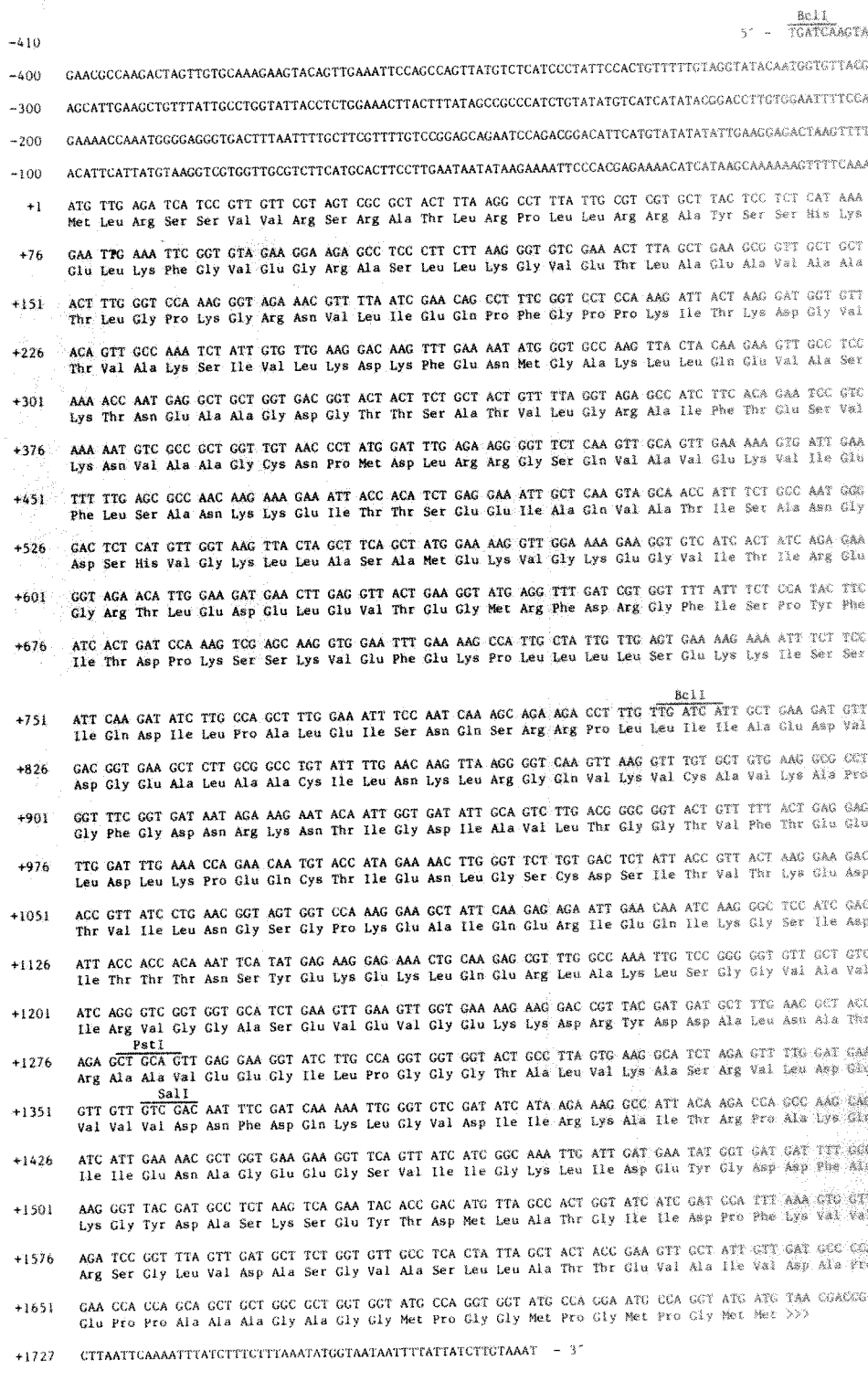


Fig. 3 Nucleotide sequence of the *S. cerevisiae* gene *HSP60*. The predicted amino-acid sequence of the hsp60 protein is indicated. The locations of *Bcl*I, *Pst*I and *Sal*I recognition sites are indicated for reference to Fig. 1.

Methods. The primary source of DNA for sequence analysis was the 5.3-kb *Eco*RI fragment shown in Fig. 1. Segments of this fragment were subcloned into pUC118 or pUC119 and sequenced by the chain-termination method[24]. Subfragments for sequence analysis were generated by exonucleolytic digestion with *Bal*31 and recloned into pUC vectors. The sequences of both DNA strands were determined, and all restriction sites used for subcloning were crossed.

Cheng *et al.*[8] have described a conditional-lethal mutation of yeast, *ts143*, which at non-permissive temperature prevents assembly of multimeric enzymes within mitochondria. To determine whether this assembly defect was due to alteration of hsp60 we tested for allelism between *ts143* and *hsp60::HIS3*. Complementation of the two mutations was assayed directly in dipolids as follows. The *ura3*$^-$/*ura3*$^-$ diploid a/αW303∇HSP60 was transformed with YEpHSP60; in subsequent manipulations the presence of extrachromosomal *HSP60* is marked by the *URA3* gene of the plasmid. Tetrad dissection gave haploids containing either functional *HSP60* or the null allele *hsp60::HIS3*, along with extrachromosomal YEpHSP60. Each type of haploid was mated to a strain containing *ts143* and diploids were selected by complementation of auxotrophies. As expected, the *HSP60/ts143* diploid frequently lost YEpHSP60 during mitotic growth, and these uracil-requiring segregants were viable at non-permissive temperature. In contrast, 100 per cent of mitotic segregants from a *hsp60::HIS3/ts143* diploid grown at non-permissive temperature were uracil prototrophs and thus contained extrachromosomal copies of *HSP60*. Therefore, *hsp60::HIS3* and *ts143* do not complement one another and, by definition, are located in the same genetic element. Using a different genetic approach Cheng *et al.*[8] independently have shown that *ts143* is an allele of *HSP60*.

Hsp60 is the second member of the heat-shock regulon shown to be required for biogenesis of mitochondrial enzymes; hsp70 proteins were recently shown to provide an 'unfolding' function necessary for translocation into both mitochondria and the

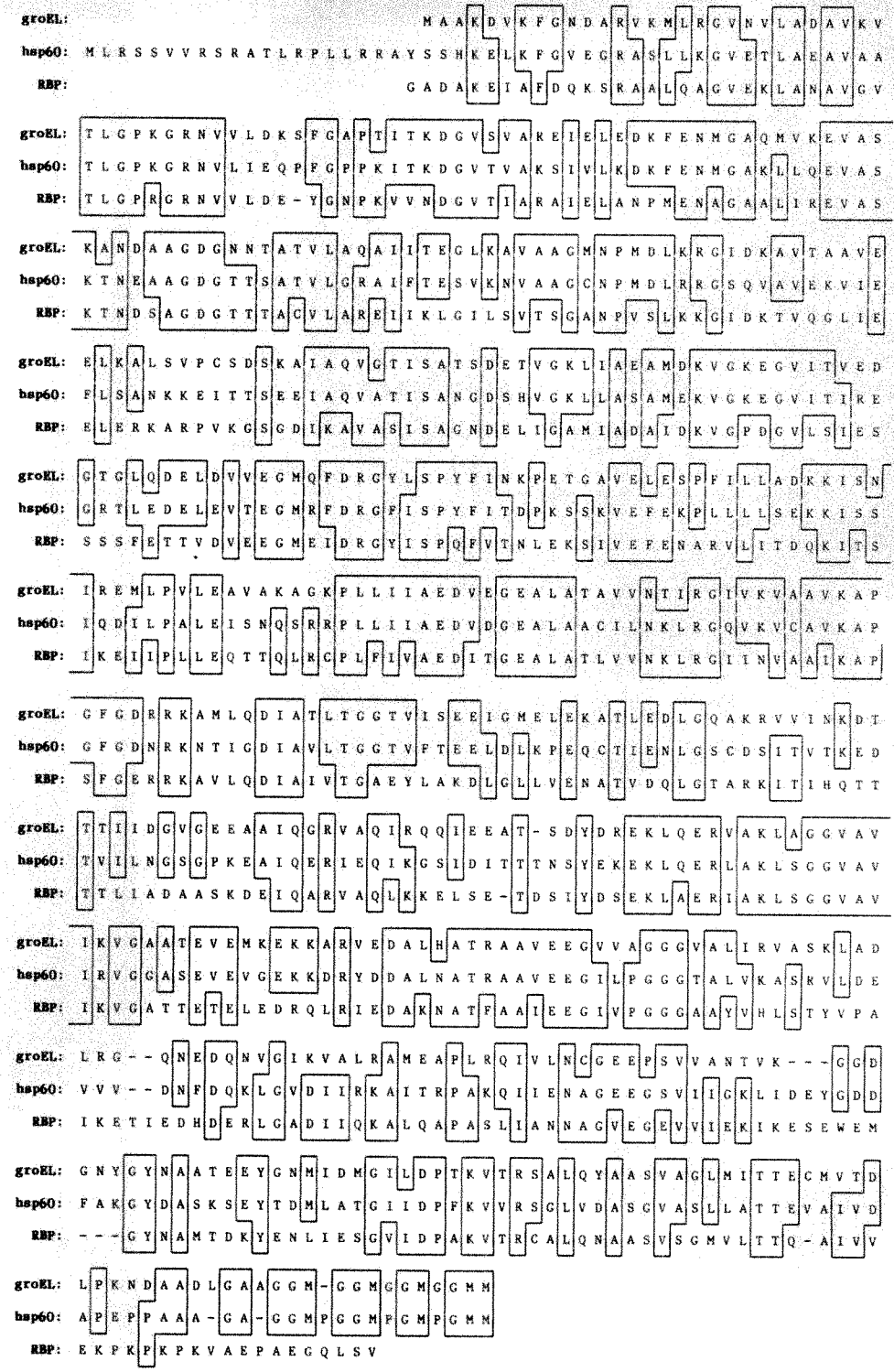


Fig. 4 Primary sequence alignment of yeast mitochondrial hsp60 with *E. coli* groEL and wheat RBP. The predicted amino-acid sequence of the *HSP60* gene product was aligned with the predicted sequences of the groEL and RBP proteins[7] using the MFALGO program[25]. Residues shared between hsp60 and either groEL or RBP are boxed.

lumen of the endoplasmic reticulum[18,19]. Thus, proteins with the ability to influence higher-order structure of various mitochondrial polypeptides are located on both sides of the organelle membrane. The accumulating evidence suggests that such 'unfoldase/refoldase' proteins are widely distributed in prokaryotic and eukaryotic cells.

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nhibition of thyroid hormone action y a non-hormone binding c-erbA rotein generated by alternative nRNA splicing

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Thyroid hormone (T3) binds to a nuclear receptor protein which egulates gene expression by binding to specific DNA sequences ear hormone-responsive genes[1]. Proteins encoded by two cellular roto-oncogenes, c-*erb*A α and β, bind T3[2,3] and can act as unctional T3 receptors[4,5]. In rats, alternative splicing of the -gene transcript generates at least two distinct protein products, ermed r-erbAα1 and r-erbAα2[5-7]. Although these proteins bind o the same DNA sequence, r-erbAα2 does not bind T3. We show ere that expression of r-erbAα2 inhibits the T3-dependent induc- ive effect of either r-erbAβ or r-erbAα1 on expression of a T3-responsive test gene. Alternative splicing of the *erb*Aα tran- cript thus generates products with opposing biological activities, uggesting a novel mechanism for the modulation of hormonal esponsiveness.

The structures of the r-erbAβ, r-erbAα1 and r-erbAα2 proteins studied here are shown schematically in Fig. 1. The human choriocarcinoma cell line JEG-3 was chosen to assess the biological activity of these proteins using contransfections of appropriate expression vectors with a T3-responsive test plasmid. As is the case with COS[4,8] and HeLa[5] cells, the addition of functional T3 receptors to JEG-3 cells by transient transfection results in a gain of hormonal responsiveness.

In the test plasmid pTK14AA, two tandem copies of a modified version of the rat growth hormone (rGH)T3-response element (TRE)[4,8] are inserted just upstream of the herpes simplex virus thymidine kinase (TK) promoter in a chloramphenicol acetyltransferase (CAT) vector[9]. As an internal control, all cotransfections also included pTKGH, in which the TK promoter directs expression of human growth hormone (hGH)[10]. Neither CAT nor hGH expression was affected by T3 when JEG-3 cells were cotransfected with just these two plasmids. CAT expression was, however, specifically induced by about 10-fold by T3 if cotransfections also included either of the r-erbA expression vectors pCDMβ or pCDMα1 (Fig. 2).

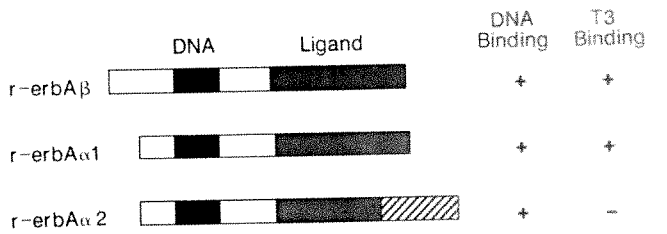


Fig. 1 Schematic representation of r-erbAα1, r-erbAα2 and r-erbAβ deduced protein sequences. DNA- and hormone-binding domains are assigned on the basis of sequence similarities with steroid receptors, and DNA- and T3-binding capacities are indicated. r-erbAα1 contains 410 amino acids, r-erbAα2 contains 492 and r-erbAβ contains 461. r-erbAα1 and r-erbAα2 are identical in their first 370 amino acids, but then diverge completely. The N-terminal regions of r-erbAα1 and r-erbAβ show no apparent similarity, whereas the putative DNA- and ligand-binding domains show 88% and 82% amino-acid identity, respectively. The βcDNA used here has been described previously[4]. The α1 cDNA used[6] is essentially identical to one isolated from rat brain[20]. The α2 cDNA used[6] is essentially identical to cDNAs isolated from rat heart[5] or brain[7], that encode proteins that do not bind T3 or its analogues. The *in vitro* translated products of all three rat erbA proteins bind specifically to the rGH TRE[5,6,21] which is also bound by T3 receptors partially purified from rat liver[22,23]. r-erbAα2 is very similar to proteins encoded by cDNAs isolated from human testis[24] and other tissues[25] which, however, are reported to bind T3. This discrepancy in hormone binding may be a consequence of several amino-acid differences in the C-terminal extensions of the rat and human proteins[26].

Cotransfection with the α2 expression vector pCDMα2 did not have a strong effect on either of the TK promoter plasmids, with or without T3. The vector pCDMα2 did, however, have a striking inhibitory effect on T3-induced expression when cotransfected with pCDMβ. Cotransfection of 5 μg pCDMα2 with 1 μg pCDMβ reduced CAT expression in the presence of T3 to nearly basal levels. α2 was a somewhat less potent inhibitor of the T3 response conferred by α1: addition of 5 μg pCDMα2 to 1 μg pCDMα1 lowered CAT expression in the presence of T3 by about 50%. A T3 dose-response study showed that the inhibitory effect of α2 was not due to an alteration in the affinity of the active T3 receptors for T3. Similar inhibitory effects of pCDMα2 were also observed in transfections of CV-1 cells.

pCDMα2 had no effect on the expression of a test plasmid analogous to pTK14AA but containing a mutant TRE. pCDMα2 also did not inhibit dexamethasone induction of a mouse mammary tumour virus promoter/CAT test plasmid when cotransfected with a glucocorticoid receptor expression vector. Thus, the inhibitory effect of α2 requires a functional TRE, and is specific for the T3 receptor.

As measured by T3 binding capacity, levels of receptor expression directed by pCDMβ and pCDMα1 were similar, and were not affected by cotransfection with pCDMα2. Nuclei of populations of JEG-3 cells transfected with either pCDM or pCDMα2 bound 9.8 fmol T3 per 100 μg DNA, confirming the inability of r-erbAα2 translated *in vitro* to bind T3[5-7]. In transfections with pCDMα1, T3 binding increased to 25.1 fmol per 100 μg DNA, and this level was unaltered by cotransfection with a fivefold excess of pCDMα2 (24.6 fmol T3 per 100 μg DNA). Nuclear T3 binding following transfection with pCDMβ was 21.3 fmol per 100 μg DNA, and 21.8 fmol per 100 μg DNA when cotransfected with a fivefold excess of pCDMα2. Assuming that <10% of the cells are transfected, these results indicate that T3 receptor levels increase by more than 20-fold in cells transfected with either pCDMβ or pCDMα1.

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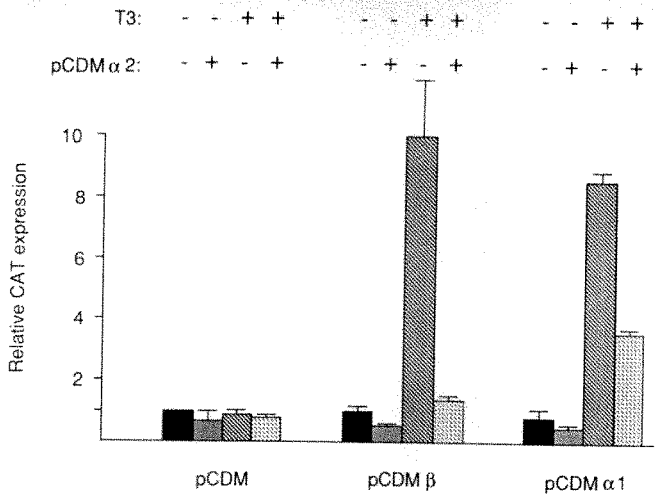


Fig. 2 r-erbAα2 inhibits T3 induction mediated by r-erbAβ or r-erbAα1. JEG-3 cells were cotransfected using $CaPO_4$ as described[4], with both 4 μg pTK14AA and 2 μg pTKGH, plus either the mammalian expression vector pCDM[27] or the indicated combinations of its r-erbA expressing derivatives. Northern blots showed that JEG-3 cells express a low level of both α1- and β-type mRNAs, but no α2-type mRNA, and confirmed that transfected cells contained levels of CDM-derived α2-mRNA comparable to the levels of CDM-derived β-mRNA present in cells transfected with pCDMβ. As observed with COS[4,8] and HeLa[5] cells, the endogenous JEG-3 receptors have no apparent effect on the activity of hormone-responsive test promoters. As results with pituitary cells suggest a correlation between the magnitude of the T3 response and receptor number[8], the lack of T3 effect may simply be a consequence of the low level of endogenous receptors. Transfections included 1 μg pCDMβ or pCDMα1, with or without 5 μg pCDMα2. pCDM was added where necessary to achieve a total of 6 μg of pCDM-based plasmids per transfection. pCDMβ is a derivative of a previously described r-erbAβ expression vector[4] containing a deletion removing all upstream AUG codons preceding the open reading frame. pCDMα1 contains a 1,799-base pair (bp) fragment beginning 13 bp upstream of the r-erbAα1[11] open reading frame, and pCDMα2 contains a 1,947-bp fragment starting at the same position and including the r-erbAα2[6] open reading frame. pTK14AA is a derivative of the CAT expression vector pUTKAT3 (ref. 9), and contains two tandem copies of a modified version of the T3 response element from the rGH gene[4,8] inserted just upstream of the TK promoter. In the internal control plasmid pTKGH[10], the TK promoter directs human growth hormone (hGH) expression. Relative CAT expression is presented in comparison to that measured in cells cotransfected with pTK14AA, pTKGH and pCDM (no *erb*A-expressing plasmids) and cultured in the absence of T3, arbitrarily defined as 1. To facilitate comparison between transfections, expression was normalized by dividing CAT activities by hGH levels as previously described[4,8,22,28].

Methods. Cells were cultured in DMEM with 10% fetal bovine serum. To measure hormone response, individual $CaPO_4$ precipitates were split into equal parts and used to transfect two plates, which were then cultured in media containing either 10% fetal bovine serum depleted of T3 using charcoal, or the same media supplemented with 5 nM T3. All such media were also supplemented with 100 nM dexamethasone. Growth of JEG-3 cells is not affected by these changes in T3 levels. After two days, CAT activity was measured in cell lysates and hGH levels were measured in media, as previously described[4]. hGH expression was essentially independent of T3 or cotransfected *erb*A plasmids, with typical concentrations ranging from 10–40 ng hGH per 100 μl media, although there was, on average, a slight decrease (approximately 30%) in hGH expression by cells transfected with functional receptors and cultured in the presence of T3. Chloramphenicol acetylation was between 1–5% for cells cultured −T3, and dilutions were made when necessary to maintain acetylation <50% for +T3 cultures. The results are the mean ±s.e.m. for three separate experiments, each consisting of transfections of two pairs of plates for all combinations of *erb*A-expressing plasmids.

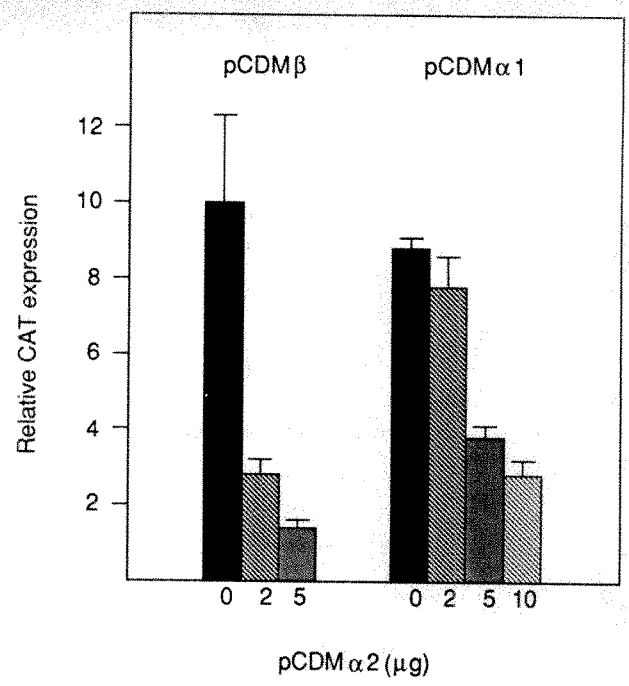


Fig. 3 pCDMα2 inhibits T3-induced CAT expression in a dose-related way. JEG-3 cells were cotransfected with 4 μg pTK14AA, 2 μg pTKGH, 1 μg of either pCDMβ or pCDMα1, and the indicated amounts of pCDMα2. Results represent relative CAT expression of cells cultured in the presence of 5 nM T3, in comparison with CAT expression of cells cotransfected with CDM alone in the absence of hormone. The experimental protocol was as described for Fig. 2.

In a dose-response study, a twofold excess of pCDMα2 ove pCDMβ decreased T3-induced CAT expression by approxi mately 70%, but higher doses of pCDMα2 were required fo similar inhibition of pCDMα1 (Fig. 3). Thus, β is more sensitiv than α1 to the inhibitory effect of α2.

To determine whether α2 had an inhibitory effect in T responsive cells, the GH4C1 rat pituitary cell line was cotransfec ted with two different rGH promoter test plasmids and increasin doses of pCDMα2. GH4C1 cells express functional T3 recep tors, and contain r-erbAα1, r-erbAβ and r-erbAα2 mRNAs[6,11] Increasing amounts of pCDMα2 strongly inhibited the relative T3 induction of the test plasmid prGH14A[8], containing the modified rGH TRE inserted upstream of the rGH promoter. As expected, prGH16A[8], which contains a deletion specifically removing the TRE, did not respond to T3 in the presence o absence of cotransfected pCDMα2.

We conclude that r-erbAα2 can inhibit T3 responses generatec by r-erbAβ and r-erbAα1. Several mechanisms could accoun for this effect: α2 could compete with the functional receptors for binding to the TRE, or could bind to the functional receptors to form inactive heterodimers or hetero-oligomers, or could compete for binding to some accessory factor required for receptor function. Given the range of ligands for the hormone receptor superfamily[12], it is interesting to speculate that the inhibitory effect of α2 could be modulated by binding of an unidentified ligand.

One specific biological role for the variant r-erbAα2 protein is suggested by the large amount of α2 mRNA in adult rat brain[6]. Although that organ has significant levels of T3 receptors, it does not respond to the hormone as judged by classic criteria[13–15]. Thus, high levels of α2 may interfere with T3 effects on expression of some genes in the brain.

Inhibition of hormone action by a splicing variant of a receptor is a novel way to modulate response. Opposing effects on gene expression have also been observed for alternatively spliced

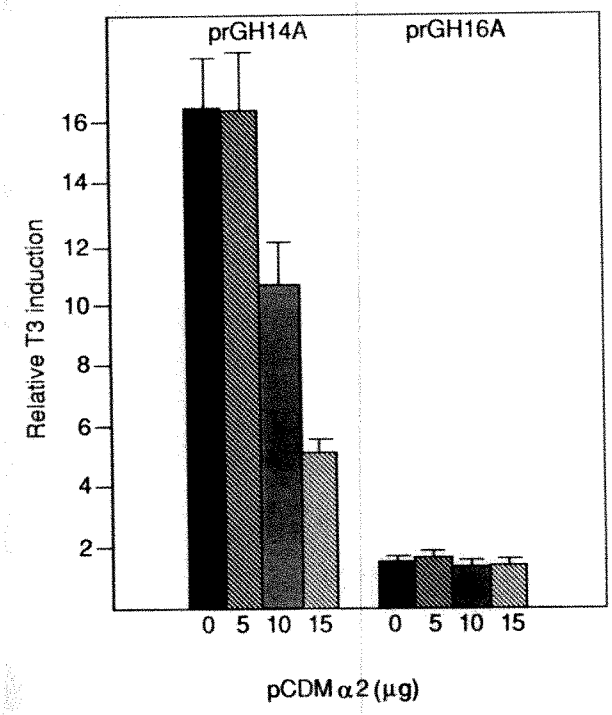


Fig. 4 pCDMα2 inhibits the activity of the endogenous T3 receptors in rat pituitary cells. GH4C1 cells were transfected using $CaPO_4$ as described[22], with 5 μg of the CAT expressing test plasmid, 3 μg of the hGH-expressing internal control plasmid, and the indicated amounts of pCDMα2. In all transfections pCDM was added as necessary to make a total of 15 μg of CDM derived plasmid per plate. Results are expressed as relative T3 induction, determined with prGH14A or pRGH16A cotransfected with pCDM, in the presence and absence of T3. prGH14A and prGH16A have been described[12] and are, respectively, derivatives of the minimal rGH promoter vector pRGH137 (refs 22, 28) containing oligonucleotide inserts of either the modified rGH TRE[4] or an inactive deletion mutant[8]. Cells were incubated and CAT and hGH were assayed as described in the Fig. 2 legend, except that the medium was Ham's F10.

products of viral genes[16,17], and recent results suggest that alternative splicing may generate diversity in the CTF/NF-1 (CCAAT-binding transcription factors/nuclear factor I) class of mammalian transcription factors[18,19]. Expression of transcription factors with different functions by alternative splicing may be an important general regulatory mechanism.

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Prolonged activation of *jun* and collagenase genes by tumour necrosis factor-α

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Tumour necrosis factor-α (TNF-α) is secreted by macrophages in response to inflammation, infection and cancer[1]. Sublethal doses of recombinant TNF-α to rats causes cachexia, anaemia and inflammation[2]. TNF-α plays a major part in tissue inflammation[3] and remodelling[4] by stimulating production of collagenase. Cellular responses to TNF-α are initiated by binding to high-affinity cell surface receptors[5,6]. TNF-α then profoundly affects gene regulation, stimulating the *fos*, *myc*, interleukin-1 and interleukin-6 genes[7–9] and inhibiting the type I collagen gene[10]. Here we demonstrate that TNF-α also stimulates collagenase gene transcription; this stimulation is mediated by an element of the gene that is responsive to the transcription factor AP-1, the major component of which (jun/AP-1) is encoded by the *jun* gene; and that TNF-α stimulates prolonged activation of *jun* gene expression. This prolonged induction of *jun* contrasts with its transient activation by the phorbol ester TPA and provides a physiological example of the ability of jun/AP-1 to stimulate its own transcription[11]. This may be a key mechanism for mediating at least some of the biological effects of TNF-α.

Addition of TNF-α to confluent human fibroblasts causes specific induction of collagenase messenger RNA (Fig. 1*a*). In these cells TNF-α does not stimulate incorporation of [^{3}H]thymidine[10], which enables us to dissociate stimulation of gene expression from a mitogenic effect. Collagenase mRNA can also be induced by TPA (12-*O*-tetradecanoylphorbol-13-acetate)[12], but not by other effectors of signal transduction, including Ca^{2+} ionophores, cyclic AMP, prostaglandins or arachidonic acid (data not shown).

Inhibition of protein synthesis (95%) by cycloheximide (0.1 mM) blocks the induction of collagenase mRNA by TNF-α (Fig. 1*b*, lane 3). By itself cycloheximide leads to low-level induction, probably by stabilizing collagenase mRNA (Fig. 1*b*, lane 4) and is not toxic to the cells. Thus *de novo* protein synthesis appears to be required for prolonged activation of collagenase gene expression by TNF-α. Transcriptional run-off assays using isolated nuclei indicate that TNF-α increases collagenase gene transcription fourfold, but that it has no effect on activity of the constitutively expressed α-tubulin gene (Fig. 1*c*). This increased collagenase gene transcription therefore apparently accounts for induction of collagenase mRNA by TNF-α.

Chimaeric plasmids containing segments of the collagenase 5′ flanking region ligated to the chloramphenicol transferase (*CAT*) gene were used to identify the TNF-α-responsive *cis*-acting element. CAT activity was first assayed in HepG2 cells, which are efficiently transfected[12] and respond to TNF-α[13]. TNF-α stimulates expression of constructs containing collagenase sequences from −1,200 to +63 or from −517 to +63

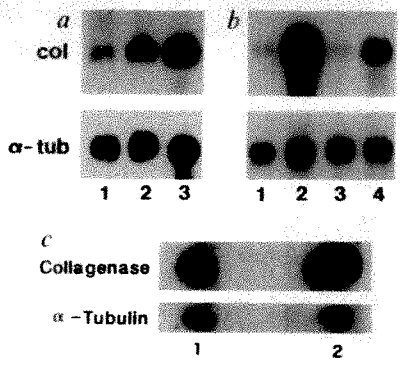


Fig. 1 Analysis of the effects of TNF-α on collagenase gene expression. *a*, Confluent primary human AF2 fibroblast cultures were incubated with human recombinant TNF-α (AmGen) (0.6 nM) for 0, 6 or 24 h (lanes 1, 2 and 3 respectively). Blots of RNA extracted from these cells were hybridized to radiolabelled complementary DNA probes of human fibroblast collagenase[12] (col) or human α-tubulin (α-tub)[33]. *b*, Confluent AF2 cultures were incubated for 24 h with no additions (lane 1); TNF-α (0.6 nM) (lane 2); TNF-α (0.6 nM) plus cycloheximide (0.1 mM) (lane 3), and cycloheximide alone (0.1 mM) (lane 4) before extraction of total cellular RNA. *c*, Nuclear run-off transcription analysis of TNF-α-stimulated collagenase gene expression in human fibroblast cultures. AF2 fibroblasts were grown to confluence in roller bottles and incubated either without (1) or with (2) TNF-α (0.6 nM) for 24 h. Nuclei were isolated and *in vitro* run-off transcription performed as previously described[9,34]. The ^{32}P radiolabelled nuclear RNA was extracted and hybridized to purified cDNAs that were slot-blotted on to nitrocellulose filters.

Methods. Human fetal AF2 fibroblasts were cultured under an atmosphere of 5% CO_2/95% air in Dulbecco's minimal essential medium (DMEM) containing 10% fetal calf serum. Cells were plated at a density of 9×10^5/P-100 dish and subjected to the various treatments after 6 days. Total cellular RNA was extracted[10]. Purified fragments of collagenase[12] and tubulin[33] cDNAs were radiolabelled with α-^{32}P-dCTP using random-primer synthesis to a specific activity of $\sim1\times10^9$ c.p.m. per μg DNA. Northern blots and slot blots on nylon filters have been described previously[10]. Slot-blot autoradiographs were quantitized by a scanning laser densitometer interfaced with an integrator.

(Fig. 2*a*); these sequences contain the TPA-responsive element (TRE) of the collagenase gene, consisting of eight base pairs (−73 to −65) constituting a binding site for transcription factor AP-1 (refs 12 and 14). TNF-α enhances the activity of constructs containing one or five copies of the TRE upstream of the herpes simplex virus-tk promoter (1×TRE-CAT, 5×TRE-CAT) by threefold and seventeenfold respectively. Neither TNF-α nor TPA stimulates the activity of tk-CAT or of Δ-72 TRE-CAT, which contains a point mutation that interferes with the binding of AP-1 to the TRE[14]. Stimulation of 5×TRE-CAT activity by TNF-α results from increased production of correctly initiated CAT mRNA (Fig. 2*c*). We confirmed these results in primary human fibroblasts, which are physiologically more relevant, but also more refractory to transfection[15]. TNF-α and TPA also stimulate CAT activity from 5×TRE-CAT, but not from tk-CAT (Fig. 2*b*), as expected. Therefore an intact AP-1 recognition sequence is necessary and sufficient for induction of CAT activity by both TNF-α and TPA in either HepG2 cells or primary human fibroblasts.

As induction of collagenase by TNF-α is dependent on *de novo* protein synthesis, we investigated whether this is associated with activation of the *jun* gene that encodes the main component of purified AP-1 known as jun/AP-1 (refs 16–18). Incubating fibroblasts with TNF-α results in prolonged induction of *jun* mRNA lasting at least six hours (Fig. 3*a*). However, the effect of TNF-α on *jun* transcription is reversible, and by 24 hours *jun* mRNA returns to its basal level (data not shown). In contrast, TPA treatment results in a more transient increase in the *jun* mRNA level, with maximum stimulation after 1 h

(*a*) CAT activity in HepG2 cells

Construct	Control	TPA	TNF-α
tk	0.2	0.2	0.4
−1,200/+63	4.0	15.3	20.4
−517/+63	0.3	2.9	3.9
1 × TRE	0.7	2.4	2.0
5 × TRE	4.7	65.3	78.8
Δ−72TRE	0.0	0.0	0.0

(*b*) CAT activity in primary fibroblasts

Construct	Control	TPA	TNF-α
tk	0.0	0.0	0.0
5 × TRE	0.2	5.1	8.2

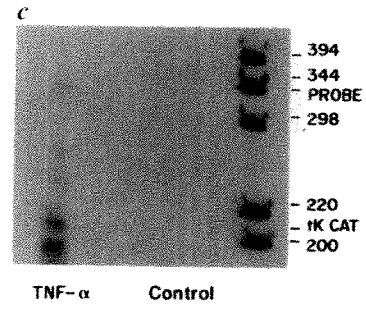


Fig. 2 Stimulation of genes containing collagenase gene segments by TNF-α. The effect of TPA and TNF-α on the chloramphenicol acetyl transferase activity of transiently transfected HepG2 cells (*a*) or AF2 primary human fibroblasts (*b*). Supercoiled plasmids (tk-CAT, −1,200/+63 collagenase-CAT, −517/+63 collagenase-CAT, 5×TRE-CAT, and Δ-72 TRE-CAT) were introduced into HepG2 cells[12] and into primary human fibroblasts using the Chen-Okayama method[15]. The cells were then incubated for 13 h either with no additions, or with TPA (20 ng ml^{-1}) or TNF-α (0.6 nM), and assayed for CAT activity[12,35]. CAT activity is expressed as per cent acetylation of chloramphenicol and represents the mean of triplicate experiments. *c*, RNase protection analysis of collagenase-CAT mRNA. HepG2 cells were transfected with 5×TRE-CAT and incubated with no additions (control) or with TNF-α (0.6 nM). Isolated total RNA (20 μg) was hybridized with 5×10^5 c.p.m. radiolabelled SP6 RNA probe[36] and digested with RNases[14]. Size markers (in base pairs) are shown to the right.

(Fig. 3*a*). Induction of *jun* mRNA by TNF-α is not blocked by cycloheximide (Fig. 3*b*), implying it does not require *de novo* protein synthesis.

As the fos protein interacts with jun/AP-1 to form a more effective transcriptional activator operating through the AP-1 binding site[18,19], we determined the effect of TNF-α on *fos* mRNA levels in these cultured fibroblasts. TNF-α or TPA ellicit a rapid and transient induction of *fos* mRNA, with virtually no *fos* mRNA being detectable by 2 h (Fig. 3*a*). The increase in *jun* and *fos* mRNA is paralleled by an increase in immunoprecipitable jun/AP-1 and fos protein synthesis (data not shown). Therefore, TNF-α stimulates the expression of both *fos* and *jun* genes, whose products interact to stimulate transcription of AP-1-responsive genes.

Most of the known effects of phorbol esters, including stimulation of gene transcription, are mediated by activation of protein kinase C (PKC)[20–22]. Activated PKC associates in a Ca^{2+}-independent manner with the plasma membrane to phosphorylate membrane proteins[21]. Two independent PKC assays demonstrated that plasma membrane-PKC activity is stimulated by incubating the fibroblasts with either TPA or TNF-α (Fig. 4). In addition, the PKC inhibitor H7 (ref. 23) inhibits induction of collagenase mRNA by either TPA or TNF-α. But prolonged coincubation with TNF-α and H7 is associated with some cel-

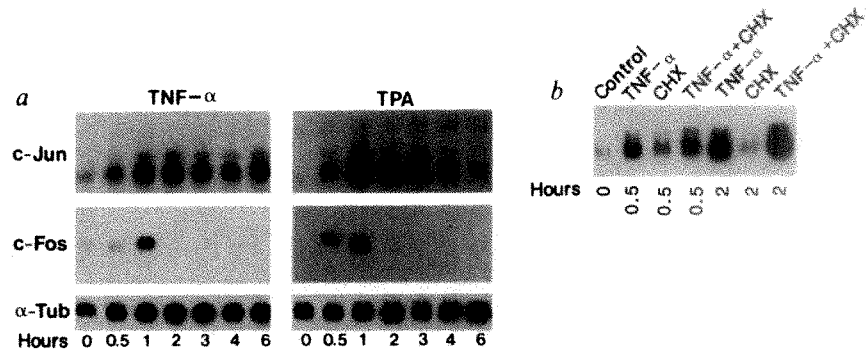


Fig. 3 Northern blot analysis of the effects of TNF-α and TPA on *jun* and *fos* expression. *a*, Confluent AF2 cultures were incubated with TNF-α (0.6 nM) or TPA (20 ng ml^{-1}) for 0, 0.5, 1, 2, 3, 4 or 6 h after which total cellular RNA was extracted. Blots of RNA from these cells were hybridized to radiolabelled *jun*[14] or α-tubulin[33] cDNA probes. *b*, Confluent AF2 cultures were incubated either with no additions (control) with TNF-α (0.6 nM), with cycloheximide (CHX; 0.1 mM) or with TNF-α (0.6 nM) plus cycloheximide (0.1 mM) for 0, 0.5 or 2 h. RNA blots were hybridized to a *jun* cDNA probe.

lular toxicity (data not shown). We conclude that like TPA, TNF-α activates PKC which in turn may result in increased AP-1 activity and induction of AP-1-responsive genes.

Epidermal growth factor[24] and serum[25] have been shown to cause transient induction of *jun* mRNA in immortal cells undergoing mitosis, which differs from the prolonged activation of gene expression in quiescent primary human fibroblast cultures shown here. Such cells are likely to reflect one of the natural targets for TNF-α action. The stimulation of *jun* and collagenase gene expression by TNF-α is prolonged in comparison to the more transient effect of TPA[11,26]. On the other hand, the kinetics of *fos* activation by both agents are identical. It is possible that TNF-α does not lead to subsequent down-regulation of PKC as rapidly as TPA (ref. 22). The *jun* gene product stimulates the transcription of its own gene through an AP-1 binding site in the *jun* promoter[11]; under certain circumstances, this positive autoregulatory mechanism could lead to subsequent prolonged stimulation of *jun* expression. It has been proposed that the autostimulatory activity of the *jun* gene plays a key part in converting transient signals generated by occupied cell surface receptors to long-lasting effects on cellular gene expression[22,27,28], a proposition which is supported by our results.

An apparent paradox is that a 'tumour necrosis factor' stimulates the expression of proto-oncogenes. In reality, TNF-α has a variety of effects on cell proliferation, including to act as a growth factor for normal fibroblasts[29] in both normal[30] and transformed[31] B cells. Bacterial extracts, such as *Bacillus Calmette-Guerin* cell-wall preparations, that stimulate TNF-α production can actually enhance tumour growth[32]. Perhaps these proliferative responses result from the stimulation of proto-oncogene expression by TNF-α.

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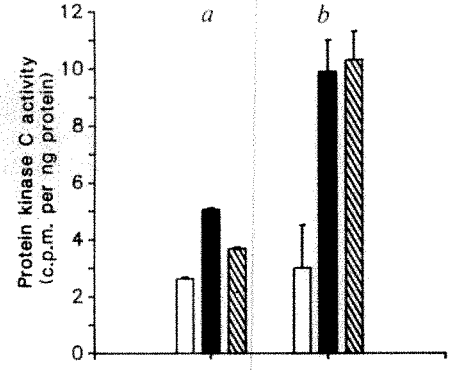


Fig. 4 Stimulation of protein kinase C activity in human fibroblast plasma membranes. Confluent AF2 cultures were incubated with no additions (control; open bars), with TPA (filled bars) or TNF-α (cross-hatched bars). Two different assays demonstrated specific activation of PKC based on its requirement for phospholipids (*a*) or its inhibition by H7 (*b*).

Methods. Confluent AF2 cells (5.6×10^6) were incubated in DMEM containing 10% fetal calf serum for 30 min at 37 °C with no additions (control) or with either TPA (20 ng ml^{-1}) or TNF-α (1.2 nM). Cells were lysed and the plasma membrane fraction purified by centrifugation at 100,000*g*. PKC was extracted from plasma membrane in 20 mM Tris, *p*H 7.5, 0.33 M sucrose, 0.5 mM EGTA, 2 mM EDTA, 2 mM PMSF and 1% NP40 and its activity determined using histone III-S as a substrate for phosphorylation[20,22]. *a*, Specific PKC activity represents ^{32}P c.p.m. incorporated into histone in the presence of phospholipids (0.9 mM diolein and 0.5 mg ml^{-1} phosphatidyl serine), minus that incorporated in their absence. *b*, Specific PKC activity represents ^{32}P c.p.m. incorporated into histone in the presence of phospholipids alone, minus that incorporated after the addition of the PKC inhibitor H7. Values represent mean ±s.e.; $P < 0.02$ for TPA and TNF-α compared with control values.

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Involvement of the 'leucine zipper' region in the oligomerization and transforming activity of human c-myc protein

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c-Myc plays a part in the regulation of important cellular processes such as growth, differentiation and neoplastic transformation[1–3]. Although c-*myc* gene structure and expression are well characterized[1–3], the function and biochemical properties of the protein are less well understood[4–6]. Human c-myc is a 439-amino acid phosphoprotein which binds DNA *in vitro* and belongs to a discrete subset of nuclear proteins[1–3]. Using the human c-*myc* mutants generated by linker-insertion and deletion mutagenesis, we have defined regions of the protein that are important for its transforming activities[7] and its nuclear localization[8]. Here, we show that human c-myc exists as an oligomer *in vitro* and use mutant proteins to localize the oligomerization domain to a carboxyl-terminal peptide containing the 'leucine zipper' motif. The 'leucine zipper' describes a structure found in a number of DNA-binding proteins that contains leucines occurring at intervals of every seventh amino acid in a region predicted to be α-helical[9]. The 'leucine zipper' might mediate dimerization by intermolecular interdigitation of the leucine side-chains. We show that a c-myc mutant, which is inactive but can oligomerize, dominantly inhibits the cotransforming activity with wild-type c-myc of rat embryo cells, whereas inactive mutants which cannot oligomerize properly because of deletions in the oligomerization domain are recessive.

To study the characteristics of c-myc, we used bacterially synthesized wild-type and mutant human c-myc in the pOTSmyc plasmid and its derivatives, produced by subcloning c-*myc* fragments containing mutations into the pOTS vector[10] (a gift from R. Watt and A. Shatzman of Smith, Kline and French). Deletion mutants are designated 'D' followed by the positions of the deleted codons. Highly purified wild-type c-myc behaves in gel filtration chromatography as a protein of relative molecular mass (M_r) 200,000 (200K) (Stokes' radius, 4.7 nm) (Fig. 1*a*). The sedimentation coefficient of c-myc is estimated to be 10.5 S by velocity sedimentation in 10–30% glycerol gradients (data not shown). The derived native relative molecular mass of c-myc is 213K, which is consistent with a tetramer[11] (Table 1). The oligomerization of c-myc was also studied by chemical cross-linking (Fig. 1*c*). The dominant cross-linked species has an estimated M_r of 200K by SDS-PAGE, which is also consistent

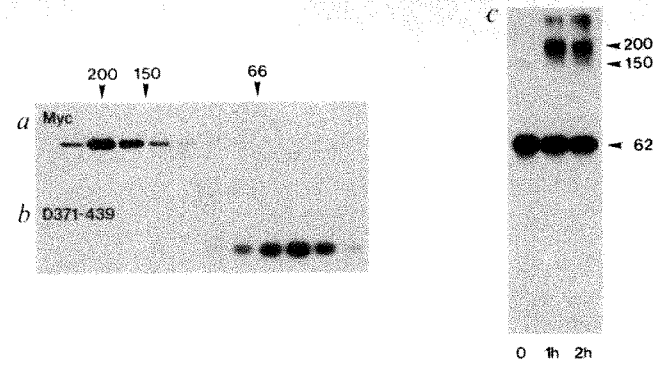


Fig. 1 Immunoblots of fractions from gel filtration chromatography of wild-type and mutant c-myc recombinant proteins. M_r values (K) for marker proteins are shown above the panels *a* and *b*: aldolase (200K), yeast alcohol dehydrogenase (150K) and bovine serum albumin (66K). *a*, The purified wild-type c-myc 62K polypeptide elutes as an oligomer of M_r 200K, whereas a mutant 52K polypeptide that is deleted from amino acids 371–439 is monomeric (*b*). *c*, Immunoblot of glutaraldehyde-cross-linked products of purified c-myc protein. M_rs of polypeptides are shown on the right (K) and incubation times (hours) at the bottom.
Methods. Mutant c-*myc* complementary DNAs in the pOTS vector were generated by subcloning *Bst*EII–*Hin*dIII fragments of mutant c-*myc* cDNAs[7] into a modified pOTSmyc vector. Recombinant wild-type and mutant c-myc protein were purified as described[16]. Protein samples (150 μl) were eluted with TBS at 6.0 ml h^{-1} at 4 °C in a 0.9 × 30 cm Sephacryl S300 (Pharmacia) column. Polypeptides in column fractions (0.5 ml) were immunoblotted after SDS-PAGE[18,19] and visualized with rabbit anti-recombinant-c-myc antibody and ^{125}I-labelled protein A. M_rs of polypeptides were estimated from prestained protein markers (Biorad). *c*, A 0.75 μM solution of purified c-myc protein was incubated with 0.005% glutaraldehyde in 75 mM sodium phosphate buffer, pH 7.0, at 20 °C. Samples were removed at different times, electrophoresed on SDS-polyacrylamide gels and immunoblotted.

Table 1 Estimated M_r and subunit structure of wild-type and mutant c-myc proteins and of staphylococcal protein A/c-myc fusions

Protein*	*s* (S)†	R_s (nm)‡	Native M_r§	Subunit M_r§	Structure
c-myc	10.5	4.7	213,000	62,000	tetramer
D106–143	8.5	4.5	169,000	54,000	tetramer
D371–412	6.2	4.0	107,000	55,000	dimer
D414–433	4.0	3.2	55,000	54,000	monomer
PA[372–439] myc	4.3	4.6	85,000	35,000	dimer
PA[262–326] myc	2.2	3.5	33,000	35,000	monomer
Protein A‖	2.1	5.0	45,000	45,000	monomer

* c-myc mutants are designated 'D' followed by position numbers of the deleted amino acids. Using the one-letter code, the carboxyl-terminal c-myc 72 amino acids are as follows, with the leucines in heptad repeat underlined: 369-ELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQAEEQKLISEEDLLRKRREQLKAKLEQLRNSCA. The 'leucine zipper' region includes leucines at residues 413, 420, 427 and 434. Protein A/c-myc fusion proteins are designated by the positions of the included c-myc amino acids in brackets.

† Sedimentation coefficients are estimated from peak fractions of 10–30% glycerol gradients in TBS (10 mM Tris-HCl, pH7, 150 mM NaCl).

‡Stokes' radii.

§ Native M_rs are estimated from the sedimentation coefficients and Stokes' radii using standard hydrodynamic equations[11]. Subunit M_rs are estimated from SDS-polyacrylamide gels (note that c-myc migrates slowly and has a predicted M_r of 49K).

‖ Values are from ref. 13.

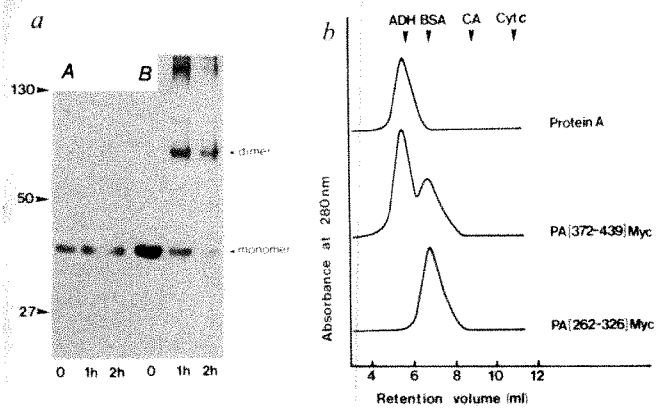


Fig. 2 *a*, Immunoblots of glutaraldehyde-cross-linked products of staphylococcal protein A/c-myc fusion proteins. The included c-myc amino acids are *A*, 262–326, *B*, 372–439. Incubation times are shown below the panels and protein size markers to the left (K). *b*, FPLC gel filtration chromatography of purified protein A/myc fusion proteins using a Superose 12 column (column volume, 25 ml). Positions of marker proteins eluting in order of decreasing Stokes' radius are given at the top: yeast alcohol dehydrogenase (ADH; R_s 4.5 nm); bovine serum albumin (BSA; R_s, 3.5 nm); bovine erythrocyte carbonic anhydrase (CA; R_s, 2.4 nm) and cytochrome *c* (Cytc; R_s, 1.7 nm).
Methods *a*, The fusion genes were constructed using the pRIT2T protein A bacterial expression vector and the fusion proteins were purified as described[12,20]. Purified proteins (1 μM) were treated with glutaraldehyde as in Fig. 1*c*. *b*, Protein samples of 200 μl (1–2 mg ml^{-1}) were injected and eluted with TBS at a flow rate of 0.5 ml min^{-1} (20 °C). Fraction volume was 0.5 ml.

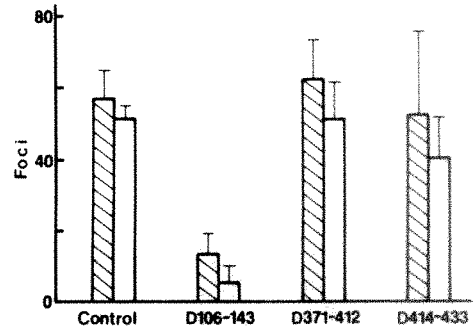


Fig. 3 Effects of defective c-myc mutants on the wild-type c-myc cotransformation activity of rat embryo cells. Rat embryo cells were transfected with EJ*ras* (10 μg) and wild-type c-*myc* (10 μg, cross-hatched; 3 μg, open) plasmids in the absence (control) or presence of various mutant c-*myc* (10 μg) plasmids[7]. The mean numbers of foci per 10^6 transfected rat embryo cells from three separate experiments are given as vertical bars with vertical lines at the top of the bars depicting standard deviations. Mutant c-*myc* plasmids alone do not cotransform rat enbryo cells with EJ*ras* (ref. 7).

with a tetrameric form; minor species migrating with M_r 150K and $M_r > 250$K are also present. The unreacted subunit runs at 62K. Thus by three independent methods, we have found that human c-myc exists as an oligomer *in vitro*.

To test whether loss of the c-myc carboxy-terminal region containing the 'leucine zipper' motif (see Table 1 footnote) prevents oligomerization, we determined the native M_rs of c-myc mutants that are deleted in this region. A mutant protein (D371–439) that is deleted at the C-terminal by 69 residues behaves as a monomeric protein of M_r 50K by gel filtration (Fig. 1*b*). To analyse this region further, we examined the gel filtration characteristics of mutant myc containing smaller deletions. D371–412 yields a 55K protein which has an M_r of slightly less than 150K in the native form, indicating the presence of dimers (Table 1). D414–433 totally disrupts the 'leucine zipper' motif and behaves as a monomeric protein with native $M_r = 55$K (Table 1). As a control, deletion of residues 106–143 (another region required for myc transforming activity[7]) does not affect oligomerization (Table 1). These gel filtration results were confirmed by velocity sedimentation. The intact D371–412 protein sediments at 6.2 S, indicative of a dimer form (Table 1), whereas D414–433 sediments at 4.0 S, indicating that monomers are present (Table 1). Thus, both our gel filtration and velocity sedimentation results indicate that disruption of the 'leucine zipper' motif generates monomeric proteins, whereas disruption of the amino-acid region 371–412, which does not involve the 'leucine zipper', still permits dimerization of myc.

If C-terminal myc amino acids are involved in oligomerization, this region might behave as a discrete functional domain. Therefore we constructed genes that fuse the 28K N-terminal region of staphylococcal protein A to regions of human myc to determine whether the C-terminal c-myc region could promote oligomerization of the hybrid protein[12]. Proteins with c-myc amino acids 262–326 or 372–439 fused to protein A were purified to >95% homogeneity. These proteins are designated PA followed by the included c-myc amino acids in brackets. Cross-linking of these fusion proteins using glutaraldehyde indicates that c-myc amino acids 262–326 do not mediate dimerization of the fusion protein (Fig. 2*a*, panel *A*). In contrast, the fusion protein containing the c-myc 'leucine zipper' yields dimers and oligomers after cross-linking (Fig. 2*a*, panel *B*). We further showed that this region of c-myc promotes oligomerization by subjecting the purified proteins to fast protein liquid chromatography (FPLC) (Fig. 2*b*). Purified protein A (45K) is an asymmetric monomeric molecule with a Stokes' radius of 5.0 nm, in agreement with our observation[13] (Fig. 2*b*). The fusion protein containing the 28K N-terminal segment of protein A and the 'leucine zipper' (PA[372–439]myc) displays two peaks which are consistent with the presence of both dimers and monomers (Fig. 2*b*). The fusion protein containing c-myc amino acids 262–326 exists exclusively as a monomer and elutes at the same position as the smaller monomeric peak of PA[372–439]myc. To confirm the subunit composition of these fusion proteins, native M_rs were estimated from Stokes' radii and sedimentation coefficients (Table 1). These results indicate that the 68 amino acids at the C-terminal of myc can mediate dimerization.

If functional c-myc is multimeric *in vivo*, then an inactive c-myc derivative or *trans*-dominant negative mutant[14] capable of interacting with wild-type polypeptide chains will be inhibitory if it causes the formation of non-functional multimers. We assessed the effects of various inactive c-myc mutants on the ability of wild-type c-myc to cotransform normal rat embryo cells with EJ-*ras* (ref. 7). Inactive mutants that are oligomerization-defective, namely D371–412 and D414–433, are recessive. But the D106–143 mutant, which lacks transforming activity but preserves the oligomerization domain, can dominantly inhibit the transforming activity of wild-type c-myc (Fig. 3). These observations support the *in vivo* significance of c-myc oligomerization and are consistent with a previous report that v-myc is oligomeric in cellular extracts[15]. Although our *in vitro* data indicate the possible existence of c-myc homo-oligomers *in vivo*, the *in vivo* c-myc oligomers could be not dimers or tetramers, but complexed with other proteins like c-jun/c-fos[16,17] for example.

In summary, we have demonstrated that c-myc synthesized in bacteria exists in oligomeric form and that oligomerization depends on the integrity of a C-terminal region containing the 'leucine zipper' motif[9]. When this region is fused to monomeric

protein A, the hybrid protein dimerizes. Another c-myc region containing amino acids 370–384 may play a part in c-myc oligomerization, because its deletion prevents tetramerization but not dimerization. As we have not performed a point mutation analysis on the 'leucine zipper', our results imply, but do not stringently prove, that the leucine repeat is the critical feature in oligomerization. The integrity of the 'leucine zipper' seems to be essential *in vivo*, as it is required for transforming activity[7] and as a defective c-myc mutant with an intact oligomerization domain behaves as a dominant negative mutant. Such a negative mutant provides a new tool to impede the activity of wild-type c-myc.

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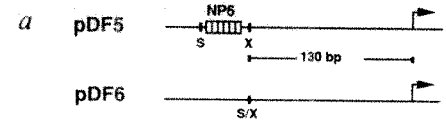
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The *Drosophila* fushi tarazu polypeptide is a DNA-binding transcriptional activator in yeast cells

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Many of the regulatory genes controlling the developmental pattern of segmentation during embryonic development in *Drosophila melanogaster*[1–3] encode nuclear proteins containing either homoeobox or 'zinc-finger' domains with putative[4–6] or demonstrated[7,8] sequence-specific DNA-binding properties. One of these *Drosophila* homoeobox-containing proteins is encoded by the *fushi tarazu* (*ftz*) gene. The expression of *ftz* is spatially restricted during embryogenesis[9–11] and the ftz polypeptide has an important role in different stages of development[12,13]. To determine whether the ftz polypeptide is a sequence-specific DNA-binding activator of transcription, we expressed portions of ftz as fusions with the yeast transcription factor GAL4 in yeast cells. Chimaeric GAL4/ftz proteins, like GAL4 itself, activated the transcription of a GAL4-dependent reporter gene. With reporter constructs containing *Drosophila*-derived chromosomal DNA sequences as transcriptional elements, the ftz polypeptide acted as a sequence-specific DNA-binding transcriptional activator. In *Drosophila*, the ftz product may therefore be a positive regulator of transcription.



b

Transcriptional activator	Transcriptional activity (β-gal units)	
	pDF5	pDF6
G/ftz$^{3-413}$	135	0.7
GAL4/ftz$^{3-413}$	103	0.5
GAL4/ftz$^{\Delta 274-302}$	0.7	<0.5
GAL4	<0.5	<0.5

Fig. 2 Consensus binding sites for ftz in *Drosophila* act as a ftz-dependent UAS element in yeast cells. Transcription of β-galactosidase reporter constructs containing either six tandem repeats (NP6) of a consensus ftz-binding site[8] in *Drosophila* (pDF5) or lacking any UAS element (pDF6) was used to assess transcriptional activation by G/ftz$^{3-413}$, GAL4/ftz$^{3-413}$, the homoeobox deletion mutant GAL4/ftz$^{\Delta 274-302}$, or GAL4 itself. *a*, Reporter constructs. *b*, Transcriptional activation.
Methods. The β-galactosidase reporter construct pDF5 containing six tandem copies of the consensus ftz-binding site TCAATTAAATGA[8] is a derivative of the 2μ *URA3* yeast plasmid pLR1Δ20 (ref. 29), in which the UAS_G DNA between the unique *Sma*1 (S) and *Xho*1(X) sites was replaced by a *Sma*1–*Sal*1 fragment from an M13mp18 clone containing the consensus ftz-binding sites. The UAS_G DNA between the *Sma*1 and *Xho*1 sites was deleted in construction of pDF6. DNA for these reporter constructs and the indicated GAL4 derivatives was used to transform the yeast strain YM709 (*Δgal80 Δgal4 ade2-101 lys2-801 his3-200 met trp tyr can1*) and β-galactosidase assays of yeast cell extracts were performed after growth on minimal medium (0.67% yeast nitrogen base without amino acids, 2% glucose) supplemented with the appropriate amino acids. The *G/ftz*$^{3-413}$ construct which expresses a chimaeric GAL4/ftz polypeptide containing amino acids 1–10 of GAL4 and amino acids 3–413 of ftz was made by introducing a 10-base pair *Eco*R1 linker at the blunted *Sph*1 site in *GAL4* DNA in the plasmid pMA235 (ref. 14) and then cloning *ftz* cDNA with *Eco*R1 linkers between the two *Eco*R1 sites of this pMA235 derivative. The non-ftz amino acids encoded at the GAL4/ftz junction of this construct are: MKLLSSIEQAEFGDP-T(3)-ftz.

To assess the transcriptional activity of ftz in yeast cells, we tested whether there were any domains within the ftz polypeptide that could functionally replace the acidic transcriptional activation domains[14] of GAL4. To do this, we fused the coding sequences of the DNA-binding domain of GAL4 (amino acids 1–147) to cDNA sequences encoding all but the N-terminal methionine and alanine residues of the ftz polypeptide. The resulting GAL4/ftz$^{3-413}$ chimaera, like similar fos and myc chimaeras[15], was almost as effective as GAL4 itself in activating transcription in yeast cells of a reporter construct containing GAL4-dependent upstream activating sequence (UAS_G) fused to β-galactosidase coding sequences (Fig. 1*a*). To map independent DNA-binding and transcriptional activation domains in the ftz polypeptide, we assessed the ability of GAL4/ftz constructs containing deletions in the ftz coding sequence to activate transcription of the same reporter gene (Fig. 1*a*). The transcriptional activation function was reduced in cells expressing deletion clones lacking the N-terminal 222 amino acids (GAL4/ftz$^{223-413}$) or C-terminal 141 amino acids (GAL4/ftz$^{3-272}$) of the ftz polypeptide and was eliminated by more extensive deletions removing the N-terminal 272 amino acids (GAL4/ftz$^{273-413}$) or the C-terminal 192 amino acids (GAL4/ftz$^{3-221}$). The inactive derivatives were not unstable poly-

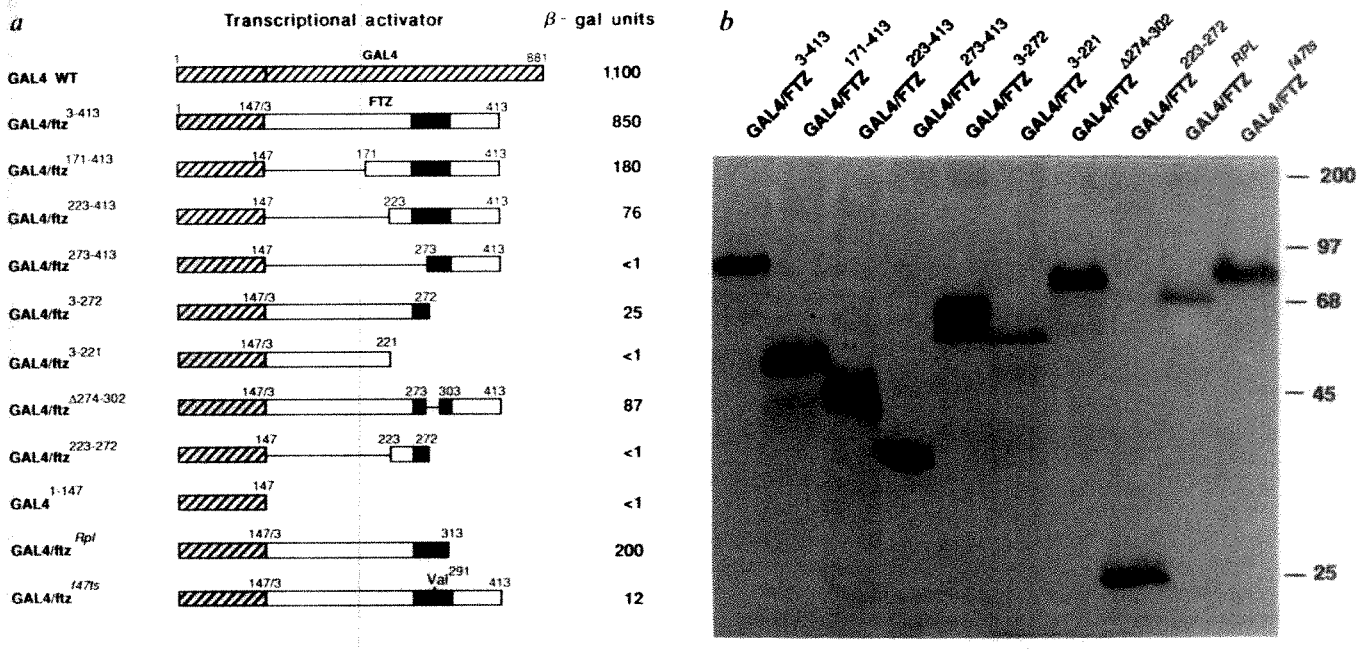


Fig. 1 *a*, UAS_G-dependent transcriptional activation by GAL4/ftz chimaeras. The ability of various GAL4/ftz chimaeric polypeptides expressed in yeast cells to activate transcription was assessed by quantitating β-galactosidase activity present in extracts of yeast cells containing a GAL4-dependent (UAS_G) β-galactosidase reporter gene. The activation of transcription by wild-type GAL4 (GAL4 WT) has been compared with that by GAL4/ftz chimaeras containing amino acids 1–147 of GAL4 and the indicated amino acids of the ftz polypeptide. The filled box denotes the homoeodomain, the hatched boxes are GAL4 sequences. *b*, Immunodetection of GAL4/ftz polypeptides present in yeast cell extracts. Relative molecular masses are shown on the right, in thousands.

Methods. Wild-type *GAL4* DNA was introduced into yeast cells on the yeast plasmid pMA210 (ref. 14). The GAL4/ftz chimaeras were constructed by cloning *ftz* cDNA fragments originating from the plasmid pGEMf1 (ref. 11) into the unique *Eco*R1 site encoding amino acid 148 of GAL4 in the pMA210 derivative pMA235 (ref. 14). GAL4 and the GAL4/ftz chimaeras were therefore expressed from the *ADH1* promoter on a *HIS3* 2μ yeast vector as described[14]. The correct reading-frame fusions were achieved using an appropriately sized *Eco*R1 linker and/or by first cloning the *ftz* DNA fragments into the polylinker of pEMBL or Bluescript vectors. The amino acids (one-letter code) at the GAL4/ftz junctions were: for GAL4/ftz^{3-413}, GAL4/ftz^{3-272}, and GAL4/ftz^{3-221}, GAL4-S(147)-PEFIKEEKLTMRDP-T(3)-ftz; for GAL4/ $ftz^{171-413}$, GAL4-S(147)-PEFELGTRGSSR-V(171)-ftz; for GAL4/$ftz^{223-413}$ and GAL4/$ftz^{223-272}$, GAL4-S(147)-PEFELGTP-V(223)-ftz; for GAL4/$ftz^{273-413}$, GAL4-S(147)-PEFELGTRGSSRV-E(273)-ftz; for GAL4/ftz^{Rpl} and GAL4/ftz^{f47ts} GAL4-S(147)-PEFGDP-T(3)-ftz. Amino acids in the C-terminal extensions before the first in-frame stop codon were: for GAL4/ftz^{3-272}, L(272)-DREGVPLQ; GAL4/ftz^{3-221}, S(221)-GL; and for $GAL4^{1-147}$, S(147)-PEF. The GAL4/$ftz^{\Delta 274-302}$ construct was made by deletion of *ftz* cDNA between its *Xho*1 and *Bgl*II sites. DNA of the GAL4 and GAL4/ftz constructs was used to transform the yeast strain YM709::RY171 (*Δgal80 Δgal4 ade2-101 lys2-801 his3-200 met trp tyr can1*) which contains a single copy of the GAL1/lacZ reporter RY171 (ref. 26) integrated at the *URA3* locus. The ability of the chimaeric constructs to stimulate expression of the reporter gene was quantitated by assessing β-galactosidase activity in the yeast cell extracts[27] after growth at 30 °C in minimal medium (0.67% yeast nitrogen base without amino acids, 2% raffinose) supplemented with the appropriate amino acids. The GAL4/ftz^{f47ts} strain was grown at 19 °C. For immunodetection of GAL4/ftz polypeptides, protein extracts were prepared as described[28] from 5 ml yeast cells grown to mid log-phase. Equivalent aliquots of each protein preparation were fractionated on an 11% polyacrylamide-SDS gel, and electrophoretically transferred on to nitrocellulose. The GAL4/ftz polypeptides were detected with a polyclonal anti-ftz antibody[11] and an alkaline phosphatase-coupled second antibody preparation (Bio-rad).

peptides in yeast; each of these different GAL4/ftz chimaeric polypeptides could be detected in extracts of yeast cells with a polyclonal anti-ftz antibody[11] (Fig. 1*b*). The transcriptional activity seen with the GAL4/ftz chimaeras with either N- or C-terminal deletions of ftz coding sequences indicated that an activation domain of the ftz polypeptide might reside between amino acids 223 and 272. A GAL4/ftz fusion containing only this small domain (GAL4/$ftz^{223-272}$) did not activate transcription however. Perhaps the transcriptional activation domain of ftz is not just a short array of contiguous amino acids as it is in GAL4 (ref. 14) and GCN4 (ref. 16).

If the ftz polypeptide is a transcriptional activator in *Drosophila*, then mutations that affect ftz function in *Drosophila* might be expected to alter the transcriptional activity of ftz in yeast. The dominant allele *Rpl* in *Drosophila* encodes a ftz polypeptide truncated at its C-terminus[4]. *Rpl* causes patchy transformations of the posterior third thoracic segment into the posterior second thoracic segment[17]. In yeast cells the GAL4/ftz^{Rpl} chimaeric polypeptide had about 25% of the transcription-activating potential of the wild-type GAL4/ftz chimaera (Fig. 1*a*). This observation is consistent with the suggestion[17] that a weakened ftz^{Rpl} polypeptide may be sufficient to define parasegmental boundaries but not sufficient to activate genes of the bithorax complex properly. We also assessed the effects in yeast of a second ftz mutation. The temperature-sensitive ftz^{f47ts} allele encodes a single alanine to valine change within the homoeodomain of the ftz polypeptide[4]. In yeast cells the chimaeric GAL4/ftz^{f47ts} polypeptide was a weak activator of transcription at high temperatures (30 °C or 37 °C; data not shown) and, somewhat unexpectedly, also at low temperatures (19 °C; Fig. 1*a*). As the ftz^{f47ts} polypeptide does function at permissive temperatures in *Drosophila* embryos, it may be stabilized by interaction with other *Drosophila* transcription factors that are not present in yeast cells.

To determine whether the ftz polypeptide could also provide sequence-specific DNA-binding activity in yeast cells, we introduced into yeast cells a β-galactosidase reporter construct that contained six tandem repeats of a *Drosophila* ftz-binding chromosomal sequence[8] as a transcriptional element (Fig. 2*a*). The results of β-galactosidase assays using this reporter con-

struct in yeast cells expressing GAL4/ftz$^{3-413}$, or a second GAL4ftz chimaera (G/ftz$^{3-413}$) containing only the N-terminal 10 amino acids of GAL4, or GAL4 are shown in Fig. 2*b*. This oligomer of consensus ftz-binding sites functioned as a ftz-dependent UAS, with GAL4/ftz$^{3-413}$ and with the G/ftz$^{3-413}$ construct. Furthermore, the integrity of the homoeobox was necessary for this transcriptional activity. The deletion construct GAL4/ftz$^{\Delta 274-302}$ which lacks 29 amino acids in the homoeobox, including its putative helix–turn–helix motif, did not activate transcription of this reporter gene. Nevertheless, this construct did provide some transcriptional activation function when UAS_G-dependent activity was monitored (Fig. 1*a*).

We have exploited the species interchangeablity of transcription factors[18–22] to establish that a *Drosophila* homoeodomain-containing regulator of embryonic development can act as a positive activator of transcription. That the transcriptional activation domains of GAL4 can be replaced by *ftz* sequences implies that GAL4 and ftz may stimulate transcription by a similar mechanism, perhaps by contacting RNA polymerase II (ref. 23) or the TATA-box factor[24] directly. Our deletion analyses (Fig. 1*a*) however, did not define any short acidic activation regions in the ftz polypeptide such as those in GAL4[14]. Transcriptional activation by the ftz polypeptide may therefore depend more on the overall secondary and tertiary structure of ftz, or on its post-translational phosphorylation[11]. In preliminary experiments (V.D.F., H. M. Krause and C.J.I., unpublished observations) we have shown that the ftz polypeptide is also highly phosphorylated when it is expressed in yeast cells. These observations raise the possibility[25] that phosphorylation of serine and threonine residues may create acidic transcriptional activation domains in transcription factors such as ftz. The differential phosphorylation of ftz during *Drosophila* embryogenesis could be a fine-tuning mechanism for its potential as a transcriptional activator.

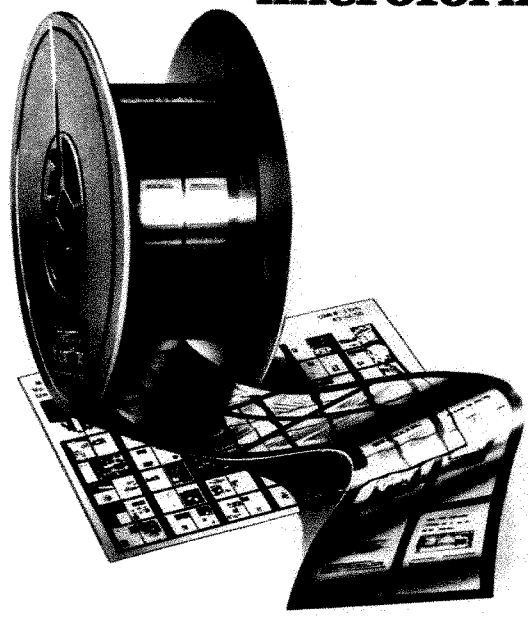
We thank Henry Krause and Jacqueline Segall for helpful suggestions, Henry Krause and Walter Gehring, Jun Ma and Mark Ptashne, Alan Laughon and Robert West for DNA, and Claude Desplans for the consensus ftz-binding element. This work was supported by grants from the MRC of Canada, the National Cancer Institute of Canada, and a grant from the NIH to the BIONET Resource. V.D.F. holds an MRC of Canada Studentship.

Note added in proof: two recent reports have demonstrated transcriptional activation by *Drosophila* homoeobox proteins[30,31].

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I.R. Krauss, P.J. Collins and S.H. Blose

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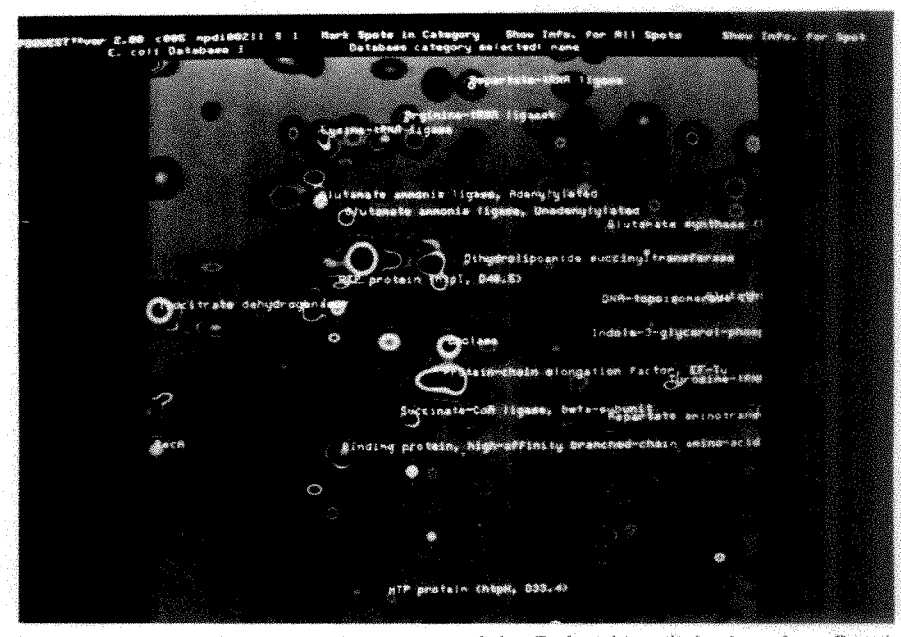


Fig. 1 Computer graphics screen showing part of the *Escherichia coli* database from Protein Databases, Inc., with several named proteins.

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Genomic activity

Cellular protein databases permit the monitoring of gene activity by detecting the expression and regulation of individual proteins, and can be used to analyse the equivalent of two million base pairs of actively coding DNA. The construction of a database of protein information produces an inventory of parts which can be used to understand how the proteins work and interact. Complementary technologies such as protein sequencing and synthesis, *in vitro* and *in vivo* genetics, and gene sequencing and synthesis may be used to extract additional information.

The collected information can then be organized into broad categories: physiologic information (regulatory behaviour and function), biochemical information (metabolic and structural data), genetic information (gene mapping and the assignment of structural genes to the 2-dimensional gel map), and architectural information (location of the protein in the cell). A database with this information permits the location of genes coding for proteins of interest, and can aid in gene cloning, and the analysis of gene networks.

As science proceeds with the sequencing of various genomes — including the human genome — the elucidation of the structure and function of proteins expressed by specific genes will allow the scientific community to understand the subtle interplay of cellular systems which lead to the regulation of organisms as a whole. The major benefit of this will be to allow the modelling of the biochemical behaviour of the cell, which should permit predictions of system behaviour and new insights into various genomes. □

Marc R. Krauss, Paul J. Collins and Stephen H. Blose are at Protein Databases, Inc., 405 Oakwood Road, Huntington Station, New York 11746, USA. For more information, fill in reader service number 100.

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Software solutions

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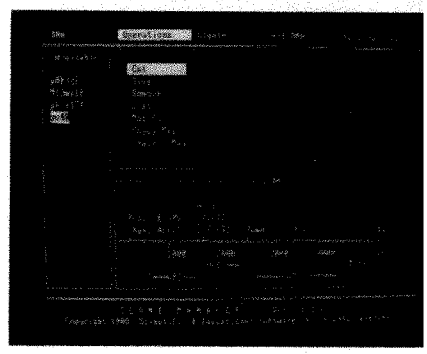
Dynamical Systems, Inc. has a set of software packages for studying **nonlinear systems and chaos** as mathematical models or as trends in experimental data gathered in any of the physical or social sciences (*Reader Service No. 101*). Dynamical Software I — now in its fourth release version — allows users to solve differential equations using an Adams-type integrator, iterate discrete maps, create 2- and 3-dimensional colour-coded phase portraits, and compute next amplitude, 1-dimensional, time-one and circle maps. Special features include data smoothing and interpolation, random number generation, and time series embedding using the Takens method. Version two of the Dynamical Software II package performs more sophisticated analyses: the integration of delay differential equations, the generation of power spectra for Rössler Attractors, and the computation of fractal dimensions, eigenvalues and eigenvectors, and Lyapunov exponents. DS I.4 costs $250 (US), and DS II.2 costs $350 (US); both can be purchased together for $550 (US). The software runs on an IBM PC/AT/XT with a 1.2 Mbyte drive and a graphics card.

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Analytical applications

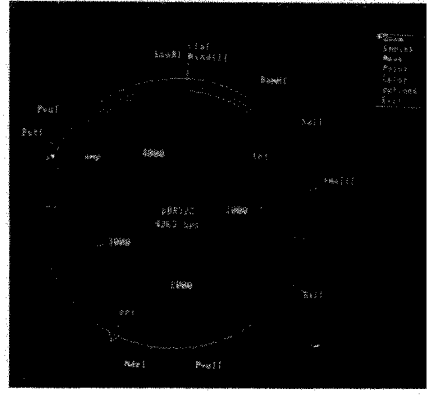
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The Clone Manager program fro Scientific & Educational Software can



The windows can be used to view linear rep sentations of the map.

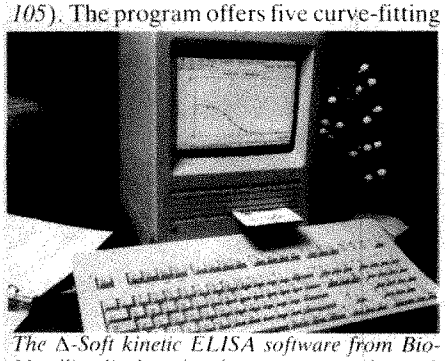
used to construct circular and linear **res riction maps** of recombinant DNA mole ules (*Reader Service No. 104*). Fra ments may be cut from any molecule a combined: the program automatical recalculates the base positions of restri tion sites, genes and markers, and dra the new map. A special "PopUp" con mand opens a window on the screen show the linear map of the DNA molecu being displayed. Cut DNA fragments a spliced together using the "Ligate" fun tion: Clone Manager automatically cognizes blunt end ligations, and w relabel a fused *Bam*HI and a *Bgl*II fra



Circular plasmid map generated using Clo Manager.

ment to acknowledge the creation of a new *Sau*3A site. The program is IBM-compatible and costs $100 (US) for individual users.

BioMetallics sells its Δ-Soft software for the collection, analysis, storage, and reporting of data from endpoint and kinetic **microtitre assays** (*Reader Service No. 105*). The program offers five curve-fitting



The Δ-Soft kinetic ELISA software from BioMetallics displays the plate pattern together with the data.

options and a complete set of statistical operations. Δ-Soft can display the plate pattern simultaneously with the corresponding data, and it has a zoom function to allow users to inspect the kinetic data on individual wells more closely. The software will perform threshold screening and masking of specific wells, and will remove outliers automatically. The Macintosh-compatible program was designed for use with Molecular Devices microtitre plate readers, but can also be used with those from several other manufacturers.

The ChemResearch software from Isco, Inc. combines **HPLC gradient programming** and system control with dual-



The Isco ChemResearch software for data management and control, along with Isco HPLC hardware.

channel data acquisition and integration (*Reader Service No. 106*). The program runs on IBM PS/2 and PC-compatible computers, and the current version supports high-resolution colour graphics and the transfer of files to other applications. Data functions include real-time display and printout, integration reports and standards comparisons. ChemResearch was written for use with Isco HPLC solvent delivery systems, but it can also be adapted for use with binary gradients and with nearly any HPLC pump which has voltage or frequency inputs.

Hitachi's Spectra Calc software interfaces Hitachi scanning UV/visible, near-infrared and fluorescence spectrophotometers with IBM PCs and compatibles (*Reader Service No. 107*). The software provides an array of commands for **spectral data processing**, including a "Draw" function which allows the use of lines, arrows, boxes, icons, and different fonts and colours for the presentation of data. Hitachi says Spectra Calc's Array Basic programming language runs at speeds associated with mini-computers, and allows users to write customized software. Files created with Spectra Calc can be converted to a variety of formats, including ASCII, Lotus, DIF and J-Camp.

Axxiom Chromatography is offering a free IBM-compatible demonstration disk for its range of PC-based **chromatography data systems** to give researchers interested in the software a chance to test it out in their own laboratory (*Reader Service No. 108*). The company offers systems which can be configured for the integration of HPLC, GC, supercritical fluid chromatography, isocratic chromatography and capillary zone electrophoresis.

Scientific publishing

Vuman Computer Systems's VuWriter **word-processing software** provides full Greek, scientific and teletex character sets, and allows the construction of brackets and integral signs of any specified height (*Reader Service No. 109*). The £95 (UK) program has an on-screen overstrike facility through which complex characters can be built, and the Greek character set has diacritics for printing Western European language characters. VuWriter can now be linked to the Aldus PageMaker and the Xerox Ventura desktop publishing systems.

EndNote, released by Niles & Associates last year, automates **bibliography building** and the formatting of in-text citations (*Reader Service No. 110*). The software is a combination of a desk accessory and an applications program, and is designed to work with Microsoft Word, MacWrite and WriteNow word-processing software. EndNote can maintain searchable databases of up to 32,000 references, and users can paste citations into text as they write, without leaving the computer to search through journals. EndNote scans the finished paper and creates a bibliography of cited works at the end of the paper, in the correct styles for *Nature*, *Science* or several other leading journals. The non-copy-protected software sells for $129 (US).

Two new updates are available for the Reference Manager **bibliography management software** from Research Information Systems (*Reader Service No. 111*). Reference Manager now has a Splicer module that can splice citations located using the software directly into word processing text, through a series of cut-and-paste operations. The Splicer module requires Reference Manager 4.0 or higher, and 210 Kbytes of available RAM after loading the word-processing program. Through the Reference Update service offered by the company, users receive a weekly diskette containing current information from over 400 of the leading biological and medical journals. The updates can be searched for combinations of authors, journal names and phrases, and identified references can be printed out as lists or reprint request cards, or can be exported to Reference Manager. Reference Manager is available for IBM-compatible and Macintosh PCs.

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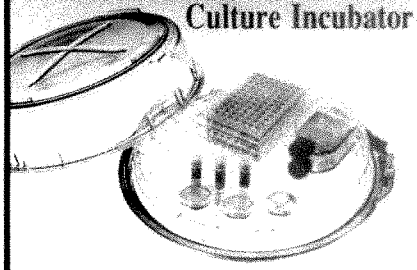
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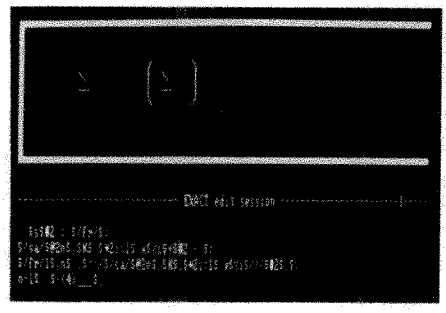
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Reader Service No. 25

The EXACT software from Technical Support Software, Inc. is for **mathematical typesetting** using Microsoft Word, WordStar, Multimate, Displaywrite, Samna, WordPerfect versions up to 5.0, and most other PC-based word-processors (*Reader Service No. 112*). EXACT is a RAM-resident program which cooperates with the user's word-processor to create complex mathematical expressions which can be displayed on the screen as they will appear in print. Through a split-screen function, EXACT can be used to edit mathematical expressions during the drafting of documents. Radicals, fractions, brackets and tables automatically rescale when they are changed: once the equation



With the EXACT mathematical software the command string and the equation can be viewed at once.

looks correct, the user can return to the text and insert the command string for printing the equation. The $495 (US) basic IBM-compatible software ($385 (US) for academics) includes 20 fonts with over 1,000 symbols and characters, plus a font editor for creating custom symbols.

Graphing and modelling

The French company Biostructure recently launched a computer-aided engineering package for evaluating the tridimensional structure of new molecules, and visualizing and manipulating them on a high-resolution graphics screen (*Reader Service No. 113*). Biostructure's BIO-EXPLORE software combines **molecular structure prediction tools** with interactive capabilities for searching the major international protein data banks to offer insights into new drug design. Protein engineers, immunologists and molecular biologists can use BIO-EXPLORE to identify homologies in known molecules and determine active sites of new molecules without performing experiments. BIO-EXPLORE is available as a turnkey system based on a Silicon Graphics workstation, starting at $81,000 (US). The software is also available as an option on Silicon Graphics and DEC workstations.

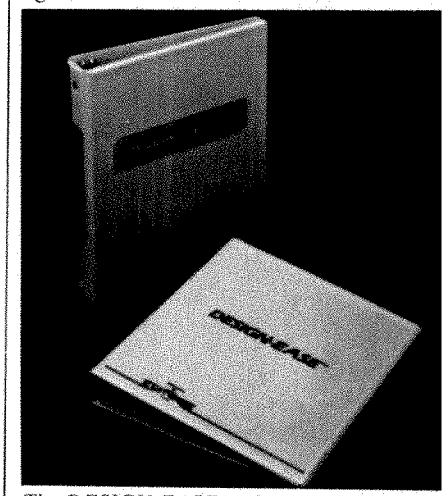
Chemical Design offers an automated procedure for the construction of **3-dimensional models of proteins**, based on software techniques developed at Birkbeck College in London (*Reader Service No. 114*). The package predicts the 3-dimensional structure of a protein by detecting homologies in its amino acid sequence with those corresponding to known protein folding patterns. Chemical Design says the approach has been used to generate useful models of proteins which shared only 20 per cent sequence homology with the protein that suggested its structure. The software matches the protein sequences and generates a number of 3-dimensional models automatically; the predicted structures can subsequently be manipulated and evaluated using molecular mechanics and dynamics programs. According to the company, the process of alignment and initial model building typically takes less than 10 minutes on a VAX 8600 computer.

New England Software has published an **X-Y graphing utility** for technical and scientific applications which it says has the same user-friendly qualities as its award-winning Graph-in-the-Box software for making business graphs (*Reader Service No. 115*). The new ANALYTIC RAM-resident software makes 16 types of graphs based on X-Y and double-Y plotting, with a choice of linear or logarithmic scaling for up to three axes. Like its Graph-in-the-Box precursor, ANALYTIC captures data directly from the screen of any application program and instantly displays the data as a graph. The program offers high-low-close graphs, error bars, curve fitting and trend analysis; the graphs can be read against both a left and right Y-axis. The $199.95 (US) IBM-compatible ANALYTIC can be used with most desktop publishing programs, and over 200 printers and plotters.

MicroMath's MINSQ package for **non-linear curve fitting and model development** can be used without data for plotting model equations, which can be optimized later to best fit data using least squares analysis (*Reader Service No. 116*). The models can be simple equations or multi-line routines that can include parametric, integral or implicit functions. Model equations can be read from a file or entered from the keyboard, then modified interactively with the built-in editor. Up to 10 data sets may be present in memory simultaneously; previous models may be recalled for further analysis or plotting. Linear parameters are identified algebraically and solved for explicitly during the model fitting process, so that by default only nonlinear parameters are defined iteratively. Subsets of data or parameters may be fitted separately before fitting the whole model, and a "fine-tuning" option allows the adjustment of step sizes and convergence criteria for difficult problems. The interactive graphics section provides facilities for zooming in on smaller regions and for annotating plots with text, lines or arrows. The non-copy-protected MINSQ requires MS-DOS 2. or greater, 512 Kbytes of compute memory and a graphics adaptor card, an costs $179 (US).

Statistics packages

Stat-Ease, Inc. is now offering site licence for its **experimental design software** pack ages, DESIGN-EASE and DESIGN



The DESIGN-EASE and DESIGN-EXPERT software packages from Stat-Ease offer statistical analysis capabilities for basic to specialized applications.

EXPERT, so that organizations can mak copies of the programs for each indivic ual's computer (*Reader Service No 117*). DESIGN-EASE uses visual graph ical techniques, and allows researchers t choose, conduct and analyse two-leve experimental designs, including fractiona factorial and Plackett-Burman designs For the advanced experimenter DESIGN-EXPERT offers response sur face and mixture design capabilities. Stat Ease says the program handles up to fiv responses simultaneously in its optimiza tion routines, and Box-Behnken an central composite designs can be con structed and analysed. Both programs ru on IBM computers and compatibles.

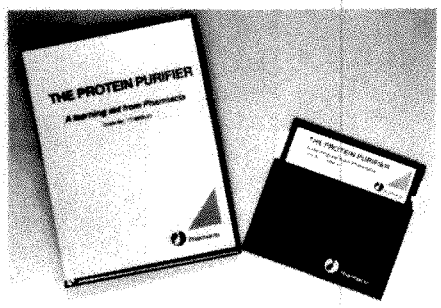
The SPSS Inc. **statistical data analysi** and presentation software is now availabl for the VAXstation 3100 and VAXstatio 3520/3540 computers (*Reader Service No 118*). SPSS-X offers a set of advanced stat istical procedures which are used in busi ness, government, and academic an scientific research. SPSS-Tables display presentation-quality results of analyses o summarized data in several tabular forms SPSS-Trends is a forecasting and time serie analysis option including Box-Jenkins an spectral analysis modeling techniques SPSS intends to develop SPSS-X softwar to support the DECstation 3100 this year The company has also signed an agree ment with Management Computer Inter national AB of Stockholm, Sweden t jointly develop a statistical data analysis package for the Apple Macintosh II and SE computers by mid-1989.

SYSTAT has just released its FASTAT **statistics and graphics package**, for the Macintosh computer (*Reader Service No. 119*). FASTAT's list of statistical feats includes one-way and multi-way tables, Chi-square, Pearson and Spearman correlations, regression, non-parametric tests, series transformation and smoothing, forecasting, principal components and common factor analysis, and travelling salesman problems. The $195 (US) software will create a sheaf of sophisticated graphs — many through only a click of the mouse — including stem-and-leaf diagrams, bubble plots, scatterplot matrices, factor-loading plots, and 2- and 3-dimensional scatterplots. MacDraw-like tools can be used to adapt and colour graphs, and scatterplot brushing tools are available for isolating and extracting cases, and "zooming in" on point clouds.

The PARVUS software package from Elsevier Scientific Software is a set of 50 programs for **multivariate statistical data analysis** (*Reader Service No. 120*). The IBM-compatible package is intended for general pattern recognition, and includes groups of programs for the explorative analysis and representation, classification and correlation of data. The programs can be divided into several functional groups: data import, data manipulation and preprocessing, methods classification, correlation analysis, target factor analysis, regression analysis, nonlinear mapping, graphical presentation, and utilities. The modular structure of PARVUS allows it to be used in a variety of ways, depending upon the application — from palaeontology to oenology. PARVUS costs $645, and comes on 15 diskettes supported by an extensive program manual.

Tutorials

The Protein Purifier from Pharmacia LKB is an IBM-based interactive program for teaching basic **protein purification techniques** (*Reader Service No. 121*). Students choose to purify one out of a starting



Pharmacia LKB's Protein Purifier tutorial program leads students through the purification process.

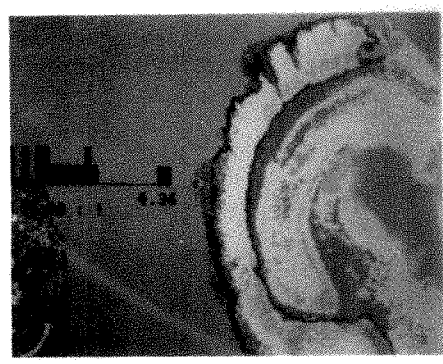
mixture of 20 proteins, using precipitation, gel filtration, hydrophobic interaction, chromatofocusing and other techniques. The software generates a chromatogram, showing the results of the separation, and efficiency tables to record how many man-hours per 100 units of international activity were required. Protein Purifier simulates activity tests to show active fractions, the pooling of tubes, and electrophoresis for determining the level or purity or certain physical characteristics.

Biosoft's Neurosim is a package of four programs which **simulate neural function** on an IBM PC (*Reader Service No. 122*). The user selects experimental and neurophysiological parameters, and the screen output resembles that of an oscilloscope during genuine experiments. Combinations of parameters can be saved as disk files, so instructors can set up experiments for subsequent demonstration. Any screen can be printed out on an Epson-compatible printer. Neurosim is comprised of four modules: Cable simulates passive conduction in the long length of an axon; HH simulates the Hodgkin-Huxley equations for the production of an action potential, and can be run clamped or unclamped; PSP shows the post-synaptic potentials developed by a neuron in response to various types of synaptic input; and Network simulates the rhythmic properties of a simple neural network of five neurons. The $199 (US) Neurosim package is for IBM PCs and true compatibles equipped with a graphics card.

TransTech has an educational/research package of software and plastic models for the investigation of **nuclear structure theory** using IBM-compatible computers (*Reader Service No. 123*). The software that comes with TransTech's face-centered cubic, or FCC, model package is designed to make quantitative comparisons among various nuclear structure models, and to elucidate the geometry of the solid-phase FCC model. The graphical displays emphasize the solid-phase FCC model and demonstrate how the liquid-drop, shell and cluster aspects of nuclei can simultaneously be found within the FCC model. TransTech says the package should also be useful for physicists involved in quark studies, because the FCC model is fundamentally a variant of the independent-particle model. TransTech sells the FCC package for $180 (US) or £90 (UK).

Image analysis

Sight Systems has developed a special software package for the automatic **analysis of autoradiographs** — the Image Manager (*Reader Service No. 124*). Image Manager offers an alternative to calibrating autoradiographs by comparing grey images with the reference exposures at the bottom of the film. With Image Manager, a video camera is used to capture an image of the autoradiograph, which is then digitized and stored on the system's 1,500-image capacity hard disk. When recalled



An autoradiograph of a rat brain processed using Image Manager, showing opiate receptors.

for analysis, the dosage levels of selected areas of the autoradiograph can be accurately determined. Greyscales reflected on the autoradiograph can be allocated pseudo colours on the screen for enhanced readability. Results can be graphed in several different formats, and can be transferred as ASCII files to other software packages for further analysis.

JAVA, from Jandel Scientific, is an IBM-compatible software package designed to **measure and analyse video images** from composite video sources, such as video cameras and VCRs, and pre-digitized formats, such as CAT, MRI and ultrasound (*Reader Service No. 125*). In addition to image processing, JAVA's capabilities include densitometry, morphometric analysis, automatic line and edge



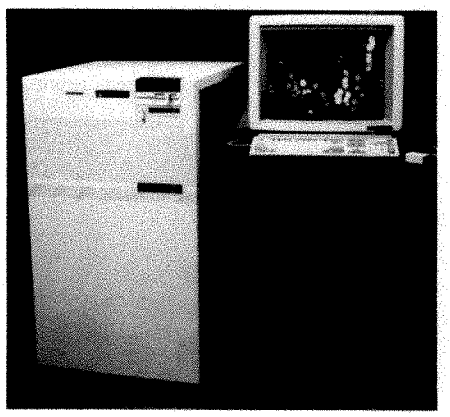
The JAVA software from Jandel measures and analyses images from a variety of sources.

digitizing, and automatic object counting. Measurements are targeted to a 64,000-row by 16,000-column virtual worksheet which includes descriptive statistical functions and data transforms. JAVA now supports both the 640-pixel and 512-pixel versions of the Imaging Technology, Inc. PCVISION*plus* line of video digitizing boards, or frame grabbers. JAVA costs $995 (US).

The HIPS **general-use image processing package** from SharpImage Software runs under the UNIX operating system, and is compatible with VAX, MicroVAX, Sun, Apollo, Masscomp, NCR Tower, Iris and IBM AT computers (*Reader Service No. 126*). HIPS handles sequences of images in the same manner in which it processes single frames. Programs have been devel-

oped for simple image transformations, filtering, convolution, Fourier and other transform processing and edge detection. Users can also integrate their own custom routines. SharpImage says the $2,995 (US) HIPS can be adapted for use with most image display devices.

The supercomputer company Stellar has announced 30 new programs for its Stellar **graphics supercomputer**, including two new packages for image processing (*Reader Service No. 127*). Stellar says its supercomputer's dynamic 3-dimensional graphics capabilities and architecture provide powerful tools for real image processing in the fields of medical imaging, seismic modelling, remote sensing and scientific visualization. The new image processing programs are the IM Raster Toolkit from the University of Waterloo for the manipulation of raster images, and the Astronomical Image Processing System from the US National Radio Astronomy Observatory. Programs for MCAE, ECAD, computational chemistry, geophysical studies, animation and computational fluid dynamics are also available.



The Stellar Graphics Supercomputer has 30 new software packages.

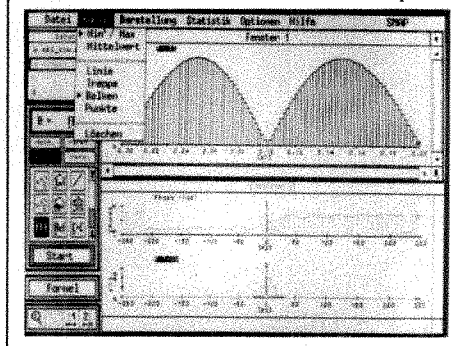
Database data

ChromHELP, Inc. offers an **HPLC/TLC database** which allows busy chromatographers to find the latest and best separation methods without searching through piles of journals (*Reader Service No. 128*). The ChromHELP database contains over 3,000 references for the separation of over 7,000 compounds, compiled from journal articles published from 1987 to 1989. All entries include title, author, references and keywords, as well as the column size and packing, the mobile phase and flow rate, and important parameters pertaining to detection of the compound. The ChromHELP database is free to purchasers of ChromHELP, Inc. columns; others pay a nominal charge for searches and printouts.

CAIRS Information Management Systems now offers a facility for immediate updating on its CAIRS **text retrieval database** for micro-to mainframe computers (*Reader Service No.129*). Until this development, new information entered on CAIRS, or modifications of existing records, were held in a holdings file and merged with the main database at periodic intervals, usually the end of the day. With the new facility, specific text in an existing record can be changed without having to go through the update process, and exposing data which must never be changed to an update procedure.

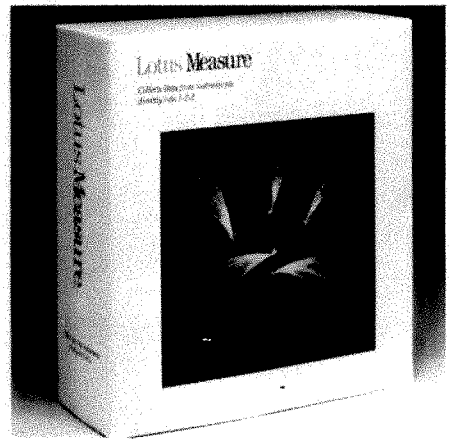
Data links

The Personal Computer Instruments-Signal Analysis Package, or PCI-SNAP, from Siemens is a **mathematical software package** for evaluating and analysing measured data from PC-based instruments (*Reader Service No. 130*). PCI-SNAP runs on any IBM-AT or XT-compatible PC with MS-DOS higher than version 2.1, and accepts data in ASCII files as well as other formats. The operation of PCI-SNAPS is graphics-oriented: the user can perform more than 100 functions and operations using the mouse, windowing, pull-down menus and graphic symbols. File management and the output of measured data is also controlled through these user interfaces.



The German version of Siemen's PCI-SNAP for the mathematical analysis of data.

The UK distributor of Lotus Measure, Industrial Data Processing Ltd, is now giving hands-on courses on how scientists and engineers can adapt the software using a PC for their own **data acquisition an analysis** applications (*Reader Service N 131*). Topics to be covered include inte facing with instruments, sensors, plug-i boards and A/D devices, real-time da analysis and display, fast Fourier tran forms, graphical presentations such trend and mimic displays, curve fitting an statistics, communicating data direct to spreadsheet, and preparation of technic data for publication. Lotus Measure co trols and monitors experiments and oth processes by sending commands to instr mentation and capturing data direct from sensors and instruments connecte through IEEE-48 bus and RS-232C seri interfaces.

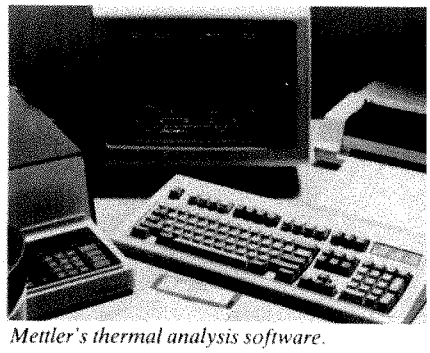


Lotus Measure can be adapted for use with scientific and engineering applications.

Dedicated programs

The Gene-Master **DNA analysis prograr** for the Gene-Master DNA Workstatio from Bio-Rad prompts the user for a parameters and sequence files required i the analysis (*Reader Service No. 132*) Version 2.0 includes the Lipman-Pearso FASTN and FASTP functions for fa alignments and similarity searches DNA and protein databases. Bio-Ra says it also locates any word in GenBan or NBRF-PIR annotations in less than fiv seconds, double-checks entries for gel entered by sonic digitizer, and insert whole sequence files into another speci fied sequence to simulate genetic engin eering experiments. The program include the Staden Shotgun Handler, whic graphically displays sequence overlaps.

The TA72 GraphWare software fror Mettler supports the processing of **thermo analytical measured values and curve** determined by the Mettler TA3000 systen (*Reader Service No. 133*). The softwar allows several thermoanalytical curves t be compared and processed simultane ously. Other features include full or par tial integration over a definable range wit different baseline types, onset determina tion with tangents, and content analysi for crystallinity.



Mettler's thermal analysis software.

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DEAN OF THE HOLCOMB RESEARCH INSTITUTE

Butler University is seeking a Dean for The Holcomb Research Institute. The Dean will be a scholar committed to the basic aims and objectives of the Institute and to the further development and teaching programs of the University.

Holcomb Research Institute, a unit of Butler University, has an endowment of $19 million and projected revenues for 1988-1989 of $2.5 million from endowment, grants, and contracts. It is housed in a modern three-story building with 50,000 sq. ft. of research space. It is presently staffed by 41 individuals, including 10 doctoral-level investigators and 10 research assistants. The Institute is a multidisciplinary, environmental research center with programs in Water Science, Biotic Resources, and Environmental Economic and Policy Analysis.

Under the terms of the Institute's charter, the Dean will lead the Institute, whose "purpose, power and function is to engage in, promote, and finance fundamental scientific research in all fields, but especially [the environmental sciences]; to train students, undergraduate and graduate, in these fields; and to disseminate the knowledge derived from the research." The Dean will develop cooperative programs and joint appointments with appropriate University departments. He or she is expected to be a senior academic worthy of appointment to the tenured faculty of one of the University's senior colleges.

Butler is an independent, non-sectarian university with a total student population of 3,912 (full-time equivalent of 2,986), located on a 280-acre campus in a residential section of one of America's most lively and livable cities. Some 60% of the students are campus residents. Enrollment is growing, with applications for 1989-1990 showing a 30% increase over the previous year. The University's long-term plan calls for significant further expansion.

Nominations and applications for the position are presently being sought, with screening to begin April 1. Applicants should submit a letter indicating interest, curriculum vitae, and the names, addresses, and phone numbers of at least three references.

Butler is an affirmative action, equal opportunity employer. Please submit applications to:

Dean Robert A. Sandmann, Chair, HRI Search Committee, Butler University, 4600 Sunset Avenue, Indianapolis, Indiana 46208.

(NW3327)A

Professor of Biological Sciences

UEA NORWICH

(Molecular biology or microbiology)

Applications are invited for a professorial appointment in the area of molecular biology or microbiology in the School of Biological Sciences. The School is seeking to appoint a candidate with a proven research record who can lead a research sector and build on the range of expertise within it. The School has very close links with two affiliated Institutes — the John Innes Institute and the Institute of Food Research, Norwich; applicants who can further research interactions with them and can complement the expertise within them will be particularly welcomed.

The appointment is available from September 1989, and the salary will be by negotiation within the professorial salary range.

Applications (five copies), giving full particulars of age, qualifications and experience, together with the names and addresses of three persons to whom reference may be made, should be lodged with the **Registrar and Secretary, University of East Anglia, Norwich, NR4 7TJ, (tel. 0603 592208)** from whom further particulars may be obtained, not later than 17 March 1989.

The University is an equal opportunities employer. (8425)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
Post-doctoral Research Assistant Pharmacologist/Biochemist

A post-doctoral position is available to study the interaction of endothelin and related peptides with neurone-like cells in culture at the biochemical level. This is an MRC position funded for three years on the Research Assistant 1A scale, available immediately.

The work will involve study of the binding of endothelin and related peptides, stimulation of release of neurotransmitter, and investigation of second messengers, regulation of intracellular Ca^{2+} etc. Experiments in the first instance will use cultured adrenal chromaffin cells.

Applications (with curriculum vitae and names and addresses of two referees) should be sent to: **Dr. M. R. Boarder, Department of Pharmacology and Therapeutics, University of Leicester, Medical Sciences Building, P.O. Box 138, University Road, Leicester, LE1 9HN, U.K. Telephone (0533) 522930.** (8417)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
LABORATORY OF PEPTIDE CHEMISTRY

A three year postdoctoral position is available immediately to work on the isolation, biosynthesis and cellular distribution of hormone processing enzymes. The position is suitable for organic chemists or biochemists; some experience in molecular biology and cell fractionation would be an advantage. Further information can be obtained from Dr D G Smyth (01-959-3666 ext 2171).

Salary will be in the range £12,520-£15,950 inclusive of London Allowance.

Applications including a curriculum vitae, and names of two professional referees, should be sent to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA,** quoting job reference: 0238/PPC. Closing date for this post will be 10th March 1989.

An Equal Opportunities Employer.

(8431)A

MRC Medical Research Council

Post-Doctoral Research Virology and Cell Biology

The Wellcome Foundation is a recognised world leader in the discovery and development of new drugs for the treatment of AIDS. Opportunities now exist within the Department of Molecular Sciences for young post-doctoral scientists to join an expanding team engaged in research on AIDS and on AIDS related diseases.

Virologist

Molecular Biology of Retroviruses

£15,000-£20,000
Beckenham, Kent

- **The Wellcome Foundation Ltd.**
- **Major International Pharmaceutical Group**
- **Research Centre at Beckenham**
- **Annual Turnover > £1,250m**
- **An Equal Opportunity Employer**

The successful candidate will be involved in an ongoing programme to monitor the drug-sensitivity of virus isolates from patients receiving therapy for HIV infection. Attempts will also be made to generate drug-resistant variants of HIV in tissue culture and to characterise the genetic defects in those viruses. In addition the appointee will also become involved in work designed to identify suitable targets for chemotherapy and this will involve investigation of the basic replication processes of the virus.

He/she should be willing to work with HIV and already have some experience in virology/molecular biology, not necessarily with HIV itself. Knowledge of virus handling, DNA manipulation and DNA sequencing would be an advantage.

This will be a 3 year post-doctoral position. (Ref. 91/54A.)

Cell Biologist

Tissue/Organ Culture

To play a key role within a new multi-disciplinary programme of research aimed at developing better chemotherapeutic agents for the treatment of Pneumocystis carinii pneumonia, a major disease within the AIDS related Complex. This new appointee would be expected to initiate and conduct research on the development of better in vitro systems for the investigation of P.carinii. He/she would already possess some experience on the initiation of highly specialised cell, organ or parasite cultures and be interested in their application to the development of in vitro drug assays against the organism. (Ref. 91/54B.)

Willingness to collaborate effectively with an integrated team of biologists, biochemists and chemists is an essential requirement for both positions.

In addition to an attractive salary, we offer an extensive range of benefits including 5 weeks' holiday, profit share scheme, subsidised restaurant, first class sports and social facilities and pension and life assurance scheme.

Please write with full career details, quoting the appropriate reference number to: Miss S. J. McKinlay, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

Wellcome

(8451)A

University of Utrecht

The department of Geology of the Faculty of Geology and Geophysics invites applications for a position in mathematical Geology.

vac.nr. 145.109

universitair docent

(postdoctoral position in mathematical geology)

The position is a temporary one, for a maximum of four years starting as soon as possible. It will be part of the faculty's geology group, which has 18 tenured staff positions covering structural and applied geology, stratigraphy, sedimentology and paleontology.

Task: To support the development of teaching and research in the geological disciplines towards the use of quantitative methods, notably in data processing and geological modelling. Teaching is at the undergraduate and graduate level. Supervision involves mainly graduate students. A primary task is to extend data processing and geological modelling into the curriculum of geology students, and to encourage working relations in this field with the Faculties of Mathematics and Information Science. Research should be directed towards interdisciplinary projects of stratigraphers, sedimentologists, structural geologists and the geophysical groups of the Faculty, with particular emphasis on the storage and processing of large data-bases concerning the study of sedimentary basin evolution and paleoenvironments, the analyses of deformation patterns and the use of remote sensing.

Require ments:
- The candidate is expected to have a Ph.D.; and a research background in seismotectonics, with a thorough knowledge of modern seismological methods applied in studies of the earthquake source.
- Willingness to cooperate in joint research projects with members of the tectonophysics, seismology and geology (mainly structural geology/tectonics) groups.

Appoint ment: The successful applicant will appointed as "Universitair Docent" at a salary in the range Dfl. 3209,— to Dfl. 6980,— for a maximum period of four years.

Information: Dr. M.J.R. Wortel, tel.: 31-30-535074
(secr.: 31-30-535086)

Application: to be sent within 14 days of publication of this advertisement to the Personnel Manager of the Institute of Earth Sciences, P.O. Box 80.021, 3508 TA Utrecht, The Netherlands.

With equally qualified applicants preference will be given to a woman.

(W5881)A

UNIVERSITY OF LEICESTER
POSTDOCTORAL RESEARCH ASSOCIATE
DEPARTMENT OF GEOGRAPHY

Applications are invited from graduates who have completed or are due to complete a Ph.D. before 30 April 1989 for a 15-month post-doctoral associateship under EEC contract No. CI.109.UK(H) "Research and control of landslides and debris flows in the loess region of Gansu Province, China". Salary will be within the range £9,865 to £15,720 depending on age and experience.

Further particulars may be obtained from **Professor E. Derbyshire, Department of Geography, to whom applications (including a C.V. and names of two referees) should be sent to arrive no later than 24 February 1989.** (8418)A

MARIE CURIE CANCER RESEARCH
RESEARCH ASSISTANTS

1. Applications are invited for a position as Research Assistant in a laboratory devoted to understanding molecular mechanisms controlling eukaryotic transcription.

   Work in this laboratory has recently identified a yeast protein with a binding site identical to that of a mammalian transcription factor. By using a sequence-specific oligonucleotide column we have purified this yeast factor to near homogeneity. The successful candidate will be expected to continue this work with a view to obtaining sufficient quantities of the yeast protein for microsequencing and for the production of antibodies for use in cloning the gene for this factor. The project will involve a wide variety of modern molecular biology techniques including assays for DNA-protein interactions, protein purification, screening cDNA libraries and site directed mutagenesis of protein coding sequences. Experience of molecular biology would therefore be an advantage.

   Enquiries and C.V.s should be addressed to Dr. C. Goding, tel. 0883-722306.

2. A Research Assistant is required for cellular and molecular studies of the biology of pigment cells which will include malignant transformation, the regulation of melanogenesis and the interactions between keratinocytes and melanocytes. Current work involves studies of transcriptional regulation of pigment-associated proteins, melanocyte transformation by U.V. light and/or recombinant retroviruses. Candidates should possess a good degree in a biological science and must have hands-on experience in cell and/or molecular biology. Enquiries to Dr. G. Currie, tel. 0883-722306.

The posts are available for 3 years in the first instance and the salary, depending upon age and experience, will be on a scale ranging from £9,865 to £15,720.

The Marie Curie Research Institute is situated on the greensand ridge near Oxted in Surrey and enjoys views over three counties. On-site low cost cottage accommodation may be available.

Applications, including C.V. and the names of two referees, to **Dr. Goding or Dr. Currie at the Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL.** (8352)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
INFECTIONS AND IMMUNITY GROUP

Applications are invited for a three year postdoctoral position in the Division of Parasitology. The project is to characterize the genes for proteins of the malaria parasite involved in the invasion of mammalian red blood cells, using rodent model systems. This is an exciting opportunity to research the molecular basis of fundamental aspects of the parasite/host relationship in malaria, a major global disease.

Candidates should have preferably a background in biochemistry or molecular biology and some experience of molecular cloning techniques. Collaboration, both within the Infections and Immunity Group and with other workers at the Institute will be actively encouraged to promote the rapid application of advances in other areas to this project.

The salary will be in the range £12,720 to £16,150 per annum, including London Allowance.

Applications in the form of a full CV a list of publications and the names of two referees should be sent to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA by 10 March 1989, quoting job reference: 0662/IPA.**

Informal enquiries to Dr K N Brown or Dr A A Holder (Tel: 01-959-3666) are welcome.

An Equal Opportunities Employer.

(8432)A

MRC
Medical Research Council

PUBLIC HEALTH LABORATORY SERVICE BOARD
PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF BIOLOGICS
TOXINOLOGY GROUP

RESEARCH TECHNICIAN (BGM)

equired to join an expanding oup concerned with research ito a variety of bacterial protein oxins. Activities of the group in- ude research into the mode of oxin action, development of uman vaccines, development of nmunoassays, and genetic anipulation studies. The success- il candidate will be required to arn and use a wide variety of chniques concerned with the nalysis and purification of pro- ins and also methods associated ith monoclonal antibody produc- on and immunoassay develop- ent. You will be required to how drive and initiative and have 1st or 2nd class honours degree r equivalent in biochemistry or a elated subject. Experience in rotein purification techniques or nmunoassay methods would be n advantage although not essen- al.

The post will be funded initially or a period of three years. Salary ill be in the range £7,825-£10,496 under review) depending on xperience.

Application forms can be btained from the Personnel Offi- er, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. el: (0980) 610391 ext. 240, to hom completed forms should be eturned by 3rd March 1989 quot- ng Post No. 0551. (8401)A

Computing Specialist with Molecular Biology Experience

We have a vacancy for a Computing Specialist to lead the Molecular Biology Database Services Section (SEQNET).

SEQNET is a national computing facility for molecular biology recently established at Daresbury to provide interactive access to databases of protein and nucleic acid sequences for academic and industrial users.

The SEQNET service is based on a Micro VAX 3600 with network connection to JANET and PSS. The centre will hold the latest versions of nucleic acid databases, protein sequence databases and associated software packages. ISIS, a combined sequence and structure database, developed by the SERC's Protein Engineering Club is also available.

• The Job

The post-holder will be a member of the Scientific Database Group and will be responsible for the quality and reliability of the SEQNET service. He/she will participate in research programmes in the Laboratory and in collaboration with external researchers.

• Qualifications and Experience

Applicants should have a relevant degree or equivalent qualification. Candidates with a 1st or 2nd class honours degree should have 2 years post-graduate research and development experience. Candidates with other qualifications should have 5 years appropriate experience.

The successful applicant will ideally be a computing molecular biologist with a good understanding of relevant applications and techniques.

Further information on the technical aspects of this post may be obtained from Dr. H. J. Sherman (0925-603339).

• Pay

Appointments will be made at Higher Scientific Officer or Senior Scientific Officer grade depending on qualifications and experience. Salary is in the range £10,026 to £17,032 with further increments depending on performance.

The superannuation scheme is non-contributory. For an application form please quote Ref. DL70 and contact:

The Personnel Officer, Daresbury Laboratory,
WARRINGTON WA4 4AD.
Tel: (0925) 603467 (24 hour answering service).

CLOSING DATE: 3rd March, 1989.

(8443)A

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

THAMES POLYTECHNIC
SCHOOL OF BIOLOGICAL SCIENCES
& ENVIRONMENTAL HEALTH

RESEARCH TECHNICIAN/ RESEARCH ASSISTANT
MOLECULAR BIOLOGY

Applications are invited for the above post which is located in the laboratory of Dr. Ian Bruce.

The School is seeking to appoint a person to work on a molecular biology project funded by Hybaid Ltd. The work will involve Southern, Northern and Western hybridization using a variety of probes and running PFG's using a range of different DNA's. Some affinity chromatography may be involved.

Ideally, applicants should hold a biology or biochemistry degree or appropriate HND/C, and have some relevant experience in some or all of these techniques. However, training will be given if necessary. Further details can be obtained by telephoning Dr. Ian Bruce on 01-316 8215.

A competitive salary will be offered in accordance with qualifications and experience.

Application is by C.V., which should be sent to the **Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF** by 3rd March, 1989.

Thames Polytechnic — seeking to achieve equality of opportunity. (8421)A

Department of Meteorology — University of Reading

Post Doctoral Research Fellow
UNIVERSITIES' GLOBAL ATMOSPHERIC MODELLING PROJECT (UGAMP)

UGAMP is a multi-institute project funded by NERC. Its aim is to increase understanding of the troposphere, stratosphere and mesosphere using advanced numerical models incorporating dynamics, radiation and chemistry, exploiting CRAY supercomputer facilities. This work is fundamental to important issues such as the greenhouse effect and threats to the ozone layer. The Fellow will assist in the extension of existing tropospheric models to the mesopause, help analyse model integrations and incorporate new processes into the model. Applicants should ideally have a doctorate in meteorology, but those with experience of numerical modelling in related disciplines should also apply. The post is currently until 31 May 1990 with salary in range £9,865-£11,680 p.a.

Apply for Application Form and further particulars to **Personnel Officer, University of Reading, Whiteknights, P.O. Box 217, Reading RG6 2AH**, telephone (0734) 318754.

Closing date 10 March 1989. Please quote Ref. R8909. (8403)A

MEDICAL RESEARCH COUNCIL COLLABORATIVE CENTRE

A collaborative research programme has been initiated at the MRC Collaborative Centre, Mill Hill, for the development of transgenic technology for the production of human therapeutic proteins.

Research will be carried out on the regulation of transgenes in mice and larger animals and on the development and exploitation of embryonic stem cell lines. The following positions are available immediately and are tenable for 3 years:

GROUP LEADER TRANSGENICS/MOLECULAR BIOLOGY

A postdoctoral scientist responsible for the day to day management of the research programme. The successful applicant will have experience of recombinant DNA techniques and the expression and regulation of mammalian genes in transgenic animals.

Salary is within the range: £16,555-£20,760 inclusive of London Allowance. Ref: CC/HTP1.

RESEARCH SCIENTISTS

Scientist with experience at the postdoctoral level in either or both of the following fields:

a) recombinant DNA technology applied to the construction of heterologous genes in mammalian cells and/or transgenic mice.

b) mammalian embryology and/or reproductive physiology.

Salary is within the range: £12,520-£15,950 inclusive of London Allowance. Ref: CC/HTP2

RESEARCH ASSISTANTS

Research Officers and/or Technicians with experience in recombinant DNA technology, cell culture and/or reproductive physiology.

Salary is within the range: £8,172-£11,311 inclusive of London Weighting. Ref: CC/HTP3.

Successful candidates will be eligible to join the MRC pension scheme.

Applications with a full curriculum vitae and the names of two referees should be sent to **Mr N J Bowry, Personnel Officer, National Institute for Medical Research, Mill Hill, London, NW7 1AA** to arrive no later than 10th March 1989. Further information about the project is available from Dr Frank Grosveld on 01-959-3666. Please quote appropriate job reference.

MRC
Medical Research Council

An Equal Opportunities Employer. (8440)A

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

GLYCOBIOLOGY UNIT

POSTGRADUATE RESEARCH ASSISTANT 1B

BR/283

Applications are invited from suitably qualified graduates for the post of Research Assistant 1B to join an active group studying the structure and function of glycoproteins.

The successful candidate will be involved in the characterization of oligosaccharides from diverse sources and in associated support duties. Previous experience is not essential, but an understanding of various chromatographic, radiolabelling and computing techniques would be an advantage.

The appointment offers promising career opportunities in this exciting field, and is available for 3 years in the first instance.

Salary on the scale £8,675–£13,365 p.a.

Applications, including a curriculum vitae and the names and addresses of 2 referees, should be sent to: **The Administrator,**

An Equal Opportunity Employer. (8412)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD,OXFORD,OX1 3QU.

THE INSTITUTE OF CANCER RESEARCH ELECTRON MICROSCOPY TECHNICIAN

The Institute of Cancer Research has a vacancy for a technician to work in its electron microscopy laboratories based at Sutton in Surrey. The laboratory provides an ultrastructural service to the Institute covering both transmission and scanning electron microscopy, with a considerable commitment to ultrastructural immunocytochemistry. In addition, the laboratory has a strong research programme on the structure of normal and neoplastic human breast epithelium.

Applicants should be qualified in biology, biochemistry or histology at 'A' level or above. Full training in all aspects of Electron Microscopy will be given.

Salary in the range of £5656 to £10711 depending on qualifications.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Further information on the post may be obtained from Dr. P. Monaghan on 01 643 8901 ext. 4631.

Applications should be sent in duplicate with the names and addresses of two referees to the **Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL quoting reference number 1.89.T.N.62.** (8394)A

UNITED MEDICAL AND DENTAL SCHOOLS LECTURESHIP IN THE DIVISON OF PHARMACOLOGY AND TOXICOLOGY

Applications are invited for a Lecturer in the Division of Pharmacology and Toxicology. The post will be for 2½ years in the first instance with a possible extension for a further 3 years.

Teaching commitments will be primarily to pre-clinical medical students and there may be some adminstrative duties. The lecturer will be expected to pursue research in pharmacology. No particular line of research is required but the division has particular interests in psychopharmacology (Guy's Campus), electrophysiology of muscle, central transmitters and retinal degenerative diseases (St Thomas's Campus). The new lecturer will be based at the campus most appropriate for his/her area of research.

Intending applicants should have a first degree in medicine, pharmacology or related subject, a PhD or MD and must have the ability to present lectures, communications etc in a clear and interesting way.

Further information may be obtained from Professor M J Neal, Division of Pharmacological Sciences, UMDS, St Thomas's Campus on 01 928-9292 extn 2080. Salary will be on the lecturer scale Grade 'A' £9,260 – £14,500 or Grade 'B' £15,105 – £19,310 plus London Weighting Allowance, depending on age and qualifications.

Applications, with full curriculum vitae (six copies) and the names of two referees, should be sent to the: **Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London, SE1 7EH, quoting reference STH/PHAR/283 by 9th March 1989.** (8397)A

UNIVERSITY OF LEICESTER
Department of Physics and Astronomy
RESEARCH APPOINTMENT IN X-RAY ASTROPHYSICS

Applications are invited for a SERC-supported post-doctoral research post in the X-ray Astronomy Group at Leicester University. The research activities of the group include observational programmes in X-ray astrophysics based on the Japanese/UK satellite GINGA, detailed preparations for the launch in February 1990 of the German/USA/UK satellite ROSAT, and the development of X-ray instrumentation for future missions such as SPECTRUM-X, AXAF and XMM.

We are interested in a person with experience in either observational astronomy, astrophysical data analysis or theoretical astrophysics. The successful candidate will be expected to develop a strong astrophysics research programme concentrating either on the facilities offered by ROSAT or ground-based support of the ROSAT programme. Some intitial commitment to the development of the software system for ROSAT data analysis may be required.

The appointment will be for up to three years. The salary, depending on age and experience, will be in the range £9,865 – £13,870 (under review) plus USS benefits.

Applications should be sent to **Professor K. A. Pounds CBE FRS, Department of Physics and Astronomy, University of Leicester, Leicester, LE1 7RH, together with a curriculum vitae and the names of two referees. The closing date is 15 March 1989.** (8416)A

Medical Writer

The success of tomorrow's technologies and products requires innovation and competence in today's research and development programmes – this is Hoechst High Chem, at the frontiers of science, technology and medicine.

Hoechst High Chem also means a highly motivated, multidisciplinary team of scientists to meet these challenges with ambition and skill in a demanding environment.

Applications are invited for the post of medical writer based at the company's headquarters in **Frankfurt.** This is a challenging opportunity for a young life scientist and native English speaker, interested in a career in medical writing and with a sound basis in the German language.

The position will involve writing and editing manuscripts for publication, and preparing in-house information and international registration dossiers.

Hoechst High Chem

Applicants should preferably be aged 25–35 and have a life science degree. Qualification to Ph. D. level would be a considerable advantage. Applicants must have the ability to express medical data accurately and concisely in English and have previous experience of writing and/or publishing scientific material.

Hoechst AG is one of the world's leading chemical companies with a very successful pharmaceutical division. The successful applicant will be attached to the Clinical Research Department and will report to the Medical Editor.

The position offers a competitive salary and a wide range of benefits including a substantial bonus and assistance with removal expenses.

If you are interested, please forward your curriculum vitae to

Hoechst AG
Personalabteilung T
Postfach 80 03 20
D-6000 Frankfurt/M. 80

Hoechst

(W5871)A

FACULTY POSITION SUNY STONY BROOK: The Department of Microbiology at SUNY Stony Brook invites applications for a tenure track or tenured position at the level of assistant, associate or full professor. The primary areas of recruitment interest are the molecular biology and genetics of eukaryotes. Preference will be given to candidates whose research relates to mechanisms of oncogenesis. Candidates must have an M.D. and/or Ph.D., postdoctoral experience and demonstrated productivity in basic research. Applicants should send a curriculum vitae, a statement of research interests and the names of three references to: **Search Committee 2, Department of Microbiology, SUNY Stony Brook, Stony Brook, NY 11794-8621.** *SUNY Stony Brook is an affirmative action/equal opportunity educator and employer.* AK25. (NW3313)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PURE AND APPLIED BIOLOGY
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post, available immediately for a fixed term of 2 years, for an investigation of the process of nematode invasion into plant roots. Specifically the project will examine the mechanism and efficiency of novel methods of nematode control. The project is funded by Shell Research.

Applicants must have a PhD in a biological subject and a proven record in research.

Salary on RA IA scale for Research and Analogous Staff within the range (£9865-£11680) according to qualifications and relevant experience.

Informal enquiries may be made to Dr Howard Atkinson (tel (0532) 332900).

Application forms and further particulars may be obtained from and completed applications forwarded to the **Registrar, The University of Leeds LS2 9TJ (tel (0532) 333963)** quoting reference number 56/24. Closing date for applications 9 March 1989. (8422)A

UNITED MEDICAL AND DENTAL SCHOOLS
(Guy's Campus)
POSTGRADUATE RESEARCH ASSISTANT – 1B

Required by the Division of Anatomy to work with a group studying the early development of the brain in higher vertebrate embryos. The successful applicant will be primarily responsible for operating a laser scanning confocal microscope and for helping in the development and application of this new technique. Technical experience not essential as training will be given. Background and interest in developmental biology and computing would be an advantage.

Starting salary up to £10,910 pa inclusive. Appointment for three years in the first instance.

Further information from Dr A G S Lumsden on 01-407 7600 ext 3359. Applications including a full CV with the names of two referees to **The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH quoting reference G/AT/289.**

Closing date 3 March. (8388)A

ROYAL HOLLOWAY AND BEDFORD
NEW COLLEGE
University of London

Cognitive Neuropsychologist/Clinical Neuropsychologist

A post doctoral or post clinical research fellow is sought in cognitive or clinical neuropsychology to develop studies of developmental disorders of cognition in collaboration with Dr Christine Temple.

The appointment will be for one year initially but extendable to three, subject to suitable progress. The post will be funded by a Research Award from the Wolfson Foundation.

Letters of application and a curriculum vitae, including the names of two referees, should be made to **Dr C Temple, Department of Psychology, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey. TW20 0EX. Applications should be made by 28th February, 1989.** (8398)A

TRANSGENE

MICROBIOLOGIST (Post Doctoral) **STRASBOURG FRANCE**

We are looking for an experienced PhD Microbiologist with a strong background in the molecular genetics of lactic acid bacteria or gram+ organisms (bacilli, corynebacteria) to join our Microbiology Division and take an active part in our research programmes involving the application of the newest genetic engineering techniques.

Interested candidates are invited to submit, in confidence, a full CV, the names and addresses of three referees, an indication of salary requirements and quoting ref. No. MD91 to:

TRANSGENE
Personnel Department
11 rue de Molsheim
67082 STRASBOURG CEDEX
FRANCE (W5875)A

DIRECTOR OF RESEARCH IN GEOLOGY

The Ecole des Mines de Paris is recruiting a Director of Research to be in charge of the Centre de Géologie Generale et Miniere.

Applicants should have experience in leading a scientific team and should be experts in applied geology (metallogeny, geochemistry, petrology, modelling, sedimentology, etc.). Preference will be given to geologists with an engineering background.

A sufficient knowledge in French is required.

Candidates should send their C.V. to the **Direction des Recherches, Ecole des Mines de Paris, 60 bd St Michel, 75272 Paris Cedex 06, before 1st March 1989.** (W5870)A

INSTITUTE OF PLANT SCIENCE RESEARCH CAMBRIDGE LABORATORY

Plant Biologist/Geneticist

The laboratory has a vacancy for a Post-doctoral scientist to join a group working on gene dispersal from transgenic plants. The aim is to assess the likelihood and consequence of transfer of genes from transgenic plants to other plants and organisms. The person appointed will be expected to transform plants, including the legume, alfalfa, and carry out glasshouse and field experiments to test the frequency of cross pollination. Experience with Rhizobium genetics would be an advantage.

The position is funded by the EEC for a period of two years.

Initially the appointee will be located at the Cambridge Laboratory but in March 1990 he or she will be re-located together with other staff to new laboratories on the John Innes site at Norwich.

Salary will be on the Higher Scientific Officer scale £10,026 p.a. to £13,460 p.a. a starting point depending on qualifications and experience.

Applications with full CV and the names of three referees to **Mrs L Cliff, Institute of Plant Science Research (Cambridge Laboratory), Maris Lane, Trumpington, Cambridge CB2 2JB quoting reference no. PB/351 by 6th March 1989.**

The Institute is an Equal Opportunities Employer (8419)A

UNIVERSITY OF CAMBRIDGE
Department of Physics
University Assistant Lecturer in Experimental Particle Physics

Applications are invited for the post of University Assistant Lecturer in the High Energy Physics Group of the Cavendish Laboratory. The research programme of the group is currently centred upon the OPAL experiment at LEP, the UA2 experiment at the CERN proton-anti-proton collider and the development of electronic detectors for particle physics.

It is hoped that the successful candidate will take up appointment by 1st October 1989, or as soon as possible thereafter. The appointment is for three years, with the possibility of reappointment for a further two years. The statutory limit of tenure of a University Assistant Lectureship is five years, but all holders of the office of University Assistant Lecturer are considered for possible appointment to the office of University Lecturer during the course of their tenure.

The pensionable scale of stipends for a University Assistant Lecturer, not ordinarily resident in College, is £10,460, rising by seven annual increments to £14,500.

Further information may be obtained from the **Secretary of the Appointments Committee for the Faculty of Physics and Chemistry, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, England,** to whom applications (ten copies), including a curriculum vitae and the names of three referees, should be sent so as to reach him not later than 31st March 1989. (8414)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF OPTHALMOLOGY
RESEARCH ASSISTANT

The post is funded by ICI Pharmaceuticals to examine the effects of the aldose reductase inhibitor, Statil, on the function and cell behaviour of retinal capillary endothelial cells and pericytes, as part of broader studies into the pathogenesis of diabetic retinopathy. The post is available for 2 years and eligible candidates should have an Honours Science Degree. The successful candidate will be encouraged to aim for a Higher Degree.

Starting salary within the range £8,675 – £11,680 on the IB Scale for Research and Analogous Staff.

Further details and application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX (tel 0224 273500) to whom applications (2 copies) should be returned by 10 March 1989 quoting reference number LW/004.** (8386)A

UNIVERSITY OF SUSSEX
SCHOOL OF BIOLOGICAL SCIENCES
NEUROPHYSIOLOGIST/CELL BIOLOGIST/ MOLECULAR BIOLOGIST/BIOPHYSICIST

Two postdoctoral fellows/research officers are required to join a research group investigating sensory processing mechanisms in the mammalian cochlea. The group works in a well equipped laboratory with good technical support and specialist training is available in a wide range of techniques.

The appointments are for three years and the salaries will be according to age and experience over the range £8,675-19,310.

Please send a CV and the names of at least two referees to **Professor I.J. Russell, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, England.**

AN EQUAL OPPORTUNITY EMPLOYER (8438)A

Molecular Science

As part of our expanding research and development programme, a new group has been established to exploit molecular biology techniques within the Division of Cellular and Molecular Sciences. The Division is engaged in a variety of drug discovery programmes covering therapeutic areas such as cancer, immunology and metabolic diseases. Two positions are vacant:

Principal Biologist

You will supervise the work of the group, which will comprise five scientists engaged in the study of gene expression using the latest technology (e.g. PCR amplification) for this purpose.

You will have several years' post-doctoral experience (or equivalent) in a related field, and proven supervisory ability. Reference G89025.

Senior Biologist

Working with the team, you will take responsibility for various sections of the project depending upon experience which is likely to include a PhD and experience in an industrial or academic environment. Reference G89026.

Both positions offer excellent remuneration packages and, where applicable, relocation assistance, together with a wide range of benefits provided by the world's largest ethical pharmaceutical company.

Please send a full c.v. or telephone for an application form by contacting Ted Smith, Senior Personnel Officer, Glaxo Group Research Ltd, Greenford Road, Greenford, Middlesex UB6 0HE. Telephone 01-422 3434, ext. 2934, quoting the appropriate reference number.

Glaxo Group Research

(8447)A

University of London

READERSHIP IN PHARMACOLOGY AT THE SCHOOL OF PHARMACY

The Senate invites applications for the above post from candidates with an appropriate record of research and teaching in pharmacology or allied subjects such as biochemistry, cell biology, immunology, physiology or pharmacy.

It is hoped to fill the Readership, vacant following the resignation of Dr A H Drummond, as soon as possible.

Applications (10 copies) should be submitted to the **Teachers' Section (N), University of London, Senate House, Malet Street, London WC1E 7HU**, from whom further particulars should first be obtained.

The closing date for receipt of applications is 31 March 1989. (8409)A

INSTITUTE OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY

FOUNDATION OF RESEARCH AND TECHNOLOGY
HERAKLION, CRETE, GREECE

POSTDOCTORAL POSITION

Applications are invited for a postdoctoral position in the insect Molecular Biology Section of the I.M.B.B. to work on the molecular genetics of hybrid dysgenesis in *Drosophila melanogaster*. The post is funded by "SCIENCE" program of the European Communities and is available for 2 years.

Candidates should be citizens of one of the member countries of the EEC (except Greece) and send their application, including a full CV and the names and addresses of two academic referees to:

Dr Christos Louis
I.M.B.B., FORTH; BOX 1527, 711 10 HERAKLION, Crete, Greece
Tel: 0030-81-238424 Fax: 0030-81-239735
E-mail: LOUIS@GRCRVAX1 (W5879)A

THE UNIVERSITY OF NEW ENGLAND
Equal Opportunity is University Policy

DEPARTMENT OF ANIMAL SCIENCE

LECTURER IN MOLECULAR GENETICS

Applicants should have a PhD or equivalent qualification with expertise in eucaryotic molecular genetics. Applicants should have experience in some aspects of recombinant DNA technology (including gene isolation and cloning, gene expression or nucleic acid sequencing), demonstrated research experience in areas of gene expression, gene regulation, gene mapping or genome organisation, and an interest in applications of molecular genetics in population/evolutionary genetics or in quantitative genetics and animal breeding.

The Department of Animal Science is responsible for all genetics teaching in the University, and the appointee will be expected to establish an independent research program, to supervise postgraduate students and to teach undergraduates; the latter with particular responsibility for a Molecular Genetics course in the Faculty of Science. The Department has a wide range of research programs in Genetics, and extensive facilities, including a fully equipped Molecular Genetics laboratory.

SALARY: Commencing salary will be determined in accordance with qualifications and experience – $A30,737-$A40,100.

CLOSING DATE: 16th March, 1989.

POSITION No.: 433.

FURTHER ENQUIRIES: Professor Barker, telephone 61 67 73 2225.

Applications should be addressed to the Staff Officer and include names and addresses of three referees and state position number. Referees should be given a copy of this advertisement and asked to write direct to the Staff Officer by the closing date and quote position number. (W5877)A

ARMIDALE, NSW 2351
AUSTRALIA

HEAD OF CELL SERVICES (ASSOCIATE SCIENTIST)

The Imperial Cancer Research Fund is one of the largest independent cancer research organisations in Europe. It is a major user of mammalian cell tissue culture, having a highly developed Central Cell Production service to support the research laboratories in the Fund's many locations in the United Kingdom, particularly London, South Mimms, Hertfordshire and Oxford.

We are seeking an experienced post-doctoral scientist to set up and run a mammalian cell tissue culture Research and Development Laboratory.

The primary aim of the laboratory will be to study various aspects of cell growth and production and downstream processing. In particular, research will be centred on the identification and development of new procedures in relation to the growth of mammalian cells in culture and the isolation and purification of cell products. This may be on one or more particular cell types, eg. monoclonal antibody producing hybridomas.

This is a demanding research appointment that will require innovative input. The successful candidate will be at post-doctoral level and have a good scientific background including experience in mammalian cell tissue culture. Particular expertise in a number of the following areas is necessary: cell metabolism, differentiated cell lines, growth factors, genetic manipulation, biochemical dynamics, growth matricies, hybridoma growth, monoclonal antibody production.

She/he will also be responsible for ensuring that the Central Cell Service is kept up-to-date with developments in methodology and technology in relation to the growth of mammalian cells and the isolation of cell products. The person appointed will also have overall responsibility for the Central Cell Service, although this will be through a Deputy Head who will be responsible for the day-to-day management of the service.

This is a new tenured research appointment and the laboratory will be based at our recently opened laboratories at Clare Hall, South Mimms, Hertfordshire.

Salary range: Associate Scientist £13,328-£21,918 inclusive.

Application should be made by sending full C.V. with names and addresses of three referees to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX quoting reference 75/R.

Smoking is actively discouraged. (8448)A

IMPERIAL
CANCER RESEARCH FUND

POSTDOCTORAL RESEARCH POSITION
Molecular/Cellular Biology

A fully funded postdoctoral research position is available immediately to study the molecular mechanisms underlying cell growth and transformation (see: Cell 49, 78-82 1987; Nature 336, 22-29 1988). Applicants should send their curriculum vitae and names of references to:

Dr. Helen Piwnica-Worms
Department of Physiology
Tufts University
136 Harrison Ave.
Boston, MA 02111 (NW3312)A

CONSULTING

U.S. scientific consulting firm offering assistance in analysis of data and communication of findings in written reports and public presentations to a variety of forums seeks Ph.D.'s in biological and biochemical sciences and M.D.'s interested in worldwide consulting opportunities. Disciplines of interest include epidemiology, biostatistics, toxicology/pharmacology, cancer research, pathology, immunology, allergy, etc., and any of the medical specialties. Applicants will provide assistance on various projects. The position is intended for those people looking to supplement ongoing activities. Travel can be minimized. Retired individuals are welcome as are all academic professionals.

Please reply with résumé and rate structure to: **Chief Executive Officer, Suite 206, 3421 M Street, N.W., Washington, DC, USA 20007.**

(NW3272)A

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND
Townsville, Australia

THE WILLARD C LACY CHAIR OF ECONOMIC GEOLOGY

After a comprehensive review of teaching and research in Geology, the University has decided to expand its activities in economic geology by creating a new Chair of Economic Geology, sponsored jointly with the exploration industry. In parallel with this initiative the University is also constructing a new research building to house applied research projects, as part of the development of a fully equipped central instrumentation facility.

The Department of Geology and its associated Economic Geology Research Unit (FGRU) constitute Australia's most concentrated research effort in economic geology. There are 18 academic staff and research fellows, and about 40 postgraduate students, who have attracted more than $1 million in external research funding in 1987-8. EGRU currently hosts a major AMIRA-funded project on epithermal gold exploration in North Queensland. Tin-tungsten exploration, structural controls on ore deposition, and coalbed methane development are other areas of research strength.

Applications are invited from suitably qualified persons for the position of Professor of Economic Geology. Applicants would be expected to possess a PhD in a relevant area, direct working experience of exploration geology and a substantial research record. The appointee will be required to vide strong academic and res leadership in Economic Ge and, as Director of EGRU, responsible for maintaining fostering relationships EGRU member companies with relevant Government professional organisations. direct knowledge of the Austr or southwest Pacific explor industry would be an advan Qualified applicants strengths in geostatistics, co ter applications in geoscien financial evaluation of mining tures are encouraged to apply University will, however, con applicants who can demons strength in any research field vant to Economic Geology.

The appointment to the C will be for a period of up to years.

Salary for the positio A$63,397 a year.

Initial enquiries may be ma confidence to Professor F Carter, Acting Director, EC on (077) 81 4536, or by FA (077) 25 1501.

Further particulars and apf tion forms are available Appointments (36010), Ass tion of Commonwealth Univ ties, 36 Gordon Square, Lo WC1H 0PF, or from the Regis James Cook University of N Queensland, Townsville, 4811, Australia, with whom a cations close on 31 March 198

Reference number 89037.

Equal Opportunity in Emp ment is University Policy (W5885)

RESEARCH ASSISTANT

Applications are invited for a Research Assistant to work on a project utilising molecular techniques to investigate the mechanisms underlying human chromosome abnormalities. The post is funded for an initial period of three years and is suitable for:

1) a Post Graduate with a first or second class honours degree. (It may be possible for such an applicant to register for a higher degree) or

2) an Individual with a Higher Degree.

Experience in Molecular Genetics is Essential. Salary £8,102-£10,496 (award pending). Enquiries to Dr Patricia A Jacobs or Dr John Harvey at the Wessex Regional Cytogenetics Unit. Telephone 0722 336212 extension 669. Application forms from the Personnel Department, Odstock Hospital, Salisbury, Wilts SP2 8BJ. Closing date: 2nd March 1989. (8371)A

THE UNIVERSITY OF T WEST INDIES
St. Augustine, Trinidad

SENIOR LECTURER/LECTURER CHEMISTRY

Applications are invited for a of Senior Lecturer/Lecturer in Department of Chemistry f physical chemist with expertis polymers and their spectrosc characterisation.

Annual salary range: Se Lecturer: TT$69,180-$90, Lecturer: TT$54,708-$77,(Pension. Passages. Hous Applications detailing qualif tions and experience and nan three referees to the Regist University of the West Indies, Augustine, Trinidad, as soor possible. Further particulars available from Appointm (36013), Association of Comm wealth Universities, 36 Gor Square, London WC1H 0PF. (W5884)

Quality in Clinical Research

"the future of successful drug development"

A major force in international pharmaceuticals my client has an enviable record for new product introduction, particularly over the last three years. With the requirements of the Regulatory Authorities becoming ever more stringent they are totally committed to ensuring that the Clinical Research process is faultless, giving new compounds the best possible chance of success.

To this end they now require a number of people to join their International Quality Assurance Group, based either in the Northern Home Counties or in Europe. Working within any part of the Clinical Research function you will be auditing and further developing Standard Operating Procedures to even higher levels of integrity.

To succeed you will have good experience in some part of the Clinical Research process, together with sharpened analytical and innovative skills that will enable you to develop new methods. You will be a qualified scientist and have the personality to influence international clinical teams. You will also be able to work alone on occasion.

If you feel ready to make a major impact on the shape of Clinical Research and the success of New Drug Approvals then send a full CV together with salary details, quoting ref **MD2074** to **Larry Botheras, Macmillan Davies, Salisbury House, Bluecoats, Hertford, SG14 1PU. Telephone (0992) 552552.**

(8433)A

Macmillan Davies

MANAGEMENT SELECTION

Senior Research Scientist

THE CELL BIOLOGY OF SHOCK AND INFLAMMATION

Celltech is one of Europe's foremost biotechnology companies; based at new, purpose-built facilities in Slough, the company is already recognised as a world-leader in the bulk production of monoclonal antibodies and recombinant proteins for diagnostic and therapeutic use.

We are now looking for an experienced PhD scientist to undertake new research into endotoxic shock and inflammation. As well as working with research colleagues from within the company, you will also enjoy collaboration with academic institutions. This is an excellent opportunity to make a real contribution to pioneering research – and to prepare for greater managerial responsibility.

You must have between three and six years' post-doctoral research experience in cell biology/immunology, ideally in a cytokine-related area, and have extensive knowledge of some aspects of inflammation. Tissue culture experience is also desirable.

We offer a competitive salary backed by an attractive benefits package, including car loan and allowance, pension scheme, free life assurance and relocation assistance if appropriate. Continuing growth at Celltech is creating many interesting and rewarding career development opportunities for our scientists.

Please write, with full details of your qualifications and experience to date, to Mrs Jane Smith, BSc C BIOL MI BIOL, at Celltech Limited, 216 Bath Road, Slough, Berkshire SL1 4EN. Please quote Ref: 237.

CELLTECH

(8450)A

INSTITUT MÉRIEUX

L'INSTITUT MERIEUX doit son développement à la qualité de sa recherche orientée vers l'application des biotechnologies à la mise au point de nouveaux produits. Dans le cadre de son expansion, il recherche un

BIOCHIMISTE

spécialisé(e) en immunologie.

Rattaché(e) au Responsable du Service de Recherche en parasitologie, il(elle) est responsable des projets de recherche qui lui sont confiés. Pour en assurer le développement, il(elle) utilise les différents procédés de purification et d'analyse. Il(elle) doit pouvoir s'imposer par sa compétence auprès de l'équipe existante.

Nous souhaitons rencontrer des candidat(e)s titulaires d'un doctorat en biochimie ou en biologie moléculaire, ayant acquis une première expérience professionnelle en laboratoire de recherche, éventuellement dans le cadre de stages post-doctoraux, et intéressé(e)s par le domaine de l'immunologie.

La maîtrise de la langue anglaise est impérative. Le poste est basé à LYON.

aum.

Merci d'adresser lettre de candidature, CV complet, photo et rémunération actuelle sous la réf. N 45/2276 G à :

EGOR BIOMEDICAL
8, rue de Berri - 75008 PARIS.

(W5889)A

EGOR

PARIS BORDEAUX LILLE LYON NANTES STRASBOURG TOULOUSE
BELGIQUE DANMARK DEUTSCHLAND ESPANA ITALIA NEDERLAND PORTUGAL UNITED KINGDOM

The University of Calgary
Research in Signal Transduction

The University of Calgary Faculty of Medicine Cell Regulation Group invites applications for faculty positions at the Assistant Professor level or higher in the area of biochemical mechanisms in signal transduction.

Successful candidates will be expected to develop an independent research program in the general area of transduction processes, but priority will be given to applicants who propose to investigate guanine nucleotide binding proteins (G-proteins) and/or phosphoinositide turnover. Well qualified candidates will be eligible to apply for salary and establishment support by competitive application to the Alberta Heritage Foundation for Medical Research. Establishment support will cover the cost of setting up a new laboratory with both major and minor equipment, and operating funds for an initial two year period.

Applicants require an MD and/or PhD and research training at the postdoctoral level. Remuneration will be based on salary scales at The University of Calgary relative to training and experience. Successful applicants will have 75% of their time protected for research.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of Calgary is committed to employment equity.

Interested candidates should submit their curriculum vitae, names of three references and a letter stating their aims and research interests by March 1, 1989 to: **Dr. David Severson, Chairman, Cell Regulation Research Group, Faculty of Medicine, The University of Calgary; 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1 Canada.** (NW3277)A

THE UNIVERSITY OF MANCHESTER
RESEARCH ASSOCIATE IN RADIO ASTRONOMY

Applications are invited for the above postdoctoral post based at the Nuffield Radio Astronomy Laboratories, Jodrell Bank. Research associates are expected to take on some service responsiblities, but at least 50% of their time will be available for their own research. The main facilities at Jodrell Bank are the 76m Lovell telescope (used for single dish work, MERLIN, and VLBI) and the seven telescope (205km) MERLIN array. Jodrell Bank has a Starlink VAX 780 and an Alliant FX/8 computer. Current areas of research include image processing, radio and optical studies of active galaxies, and quasars, gravitational lenses, neutral hydrogen studies of galaxies, molecular lines, stars, and the microwave background.

Appointment tenable from October 1st, 1989, initially for a period of 2 years with possible extension up to 3 years (maximum).

Salary range £9,865 – £15,720 p.a. depending on age and experience.

Further enquiries to, and application forms from, **I. W. A. Browne, University of Manchester, Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire, SK11 9DL. Closing date May 1st, 1989.**

The University is an equal opportunities employer (8390)A

Imperial College of Science Technology and Medicine
University of London

Condensed Matter Theorist

Applications are invited for two post-doctoral positions held in the physics department. The appointees, to commence on or before 1st October 1989, at a salary on the 1A scale (£9,865 to £15,720) plus London allowance of £1,650.

One post concerns electronic properties of solids and their surfaces and is for a period of 5 years with the possibility of further renewal. The other is for work on the structure of disordered surfaces and is for a period of 3 years. The Imperial College solid state physics theory group, one of the largest in the UK, has wide interests ranging from surfaces, to transport in disordered systems, and spin glasses.

Applicants, who will have a PhD or expect to obtain one during 1989, are asked to send a curriculum vitae and list of publications. Three referees should be asked to write directly. For further information contact the head of the solid state theory group, **John Pendry, The Blackett Laboratory, Imperial College, London, SW7 2BZ, UK. (tel 01-589-5111 x6902).** Closing date: 31st March 1989. (8426)A

PUBLIC HEALTH LABORATORY SERVICE BOARD
PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF BIOTECHNOLOGY

MOLECULAR GENETICIST

A research assistant is required to assist with work encompassing a wide variety of recombinant DNA techniques, as applied to procaryotic microorganisms. The successful candidate will join a well established team of scientists actively engaged in the application of recombinant methodology to products and processes of biotechnological interest. Basic laboratory experience and a degree, or equivalent, in a biological discipline would be an advantage.

Appointment will be made on the Basic Grade Microbiologist scale: £6,993-£10,496 (under review), for a period of 2 years.

NHS terms and conditions.

Candidates seeking further information should contact Dr N.P. Minton, tel: (0980) 610391, ext. 562.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Tel: (0980) 610391 ext. 240, to whom completed forms should be returned by 3rd March 1989 quoting Post No. 0897. (8400)A

UNIVERSITY OF CANTERBURY
New Zealand

LECTURER IN ZOOLOGY
(Aquatic Science)

Applications are invited for the above position in the Department of Zoology in the area of aquaculture which could include, for example, areas such as animal cultivation systems, controlled breeding, enhancement of natural populations, and other areas of applied aquatic zoology. The appointee would need to have skills in experimental ecology, biometrics and be able to develop courses and research in aquaculture.

The salary for Lecturers is on a scale from NZ$35,000 to NZ$42,500 (bar), NZ$44,000 to NZ$46,000 per annum.

Further particulars and Conditions of Appointment may be obtained from Appointments (36014), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Applications close with A.W. Hayward, Registrar, University of Canterbury, Private Bag, Christchurch, New Zealand, on 31 March 1989. (W5882)A

MIDLAND CENTRE FO
NEUROSURGERY AN
NEUROLOGY

HOLLY LANE, SMETHWI
WARLEY, WEST MIDLAN
B67 7JX

NEUROPHYSIOLOGIST
RESEARCH TECHNICIA
(in the Department of Neurosurg

This post will be held in the Dep
ment of Neurosurgery at the M
land Centre for Neurosurgery
Neurology. The successful ap
cant will participate in a three y
programme funded by the W
Midlands Regional Health Au
ority.

The work involves neurophy
logical monitoring of patie
undergoing a variety of proced
including functional and ster
tactic surgery. The department
a particular interest in disorder
movement. The candidate sho
have a degree in physiology
appropriate basic discipline
some experience in neurophy
logical recording techniques.

Salary scale will be £9,3
£12,347 per annum.

Application form and
Description available from
Senior Administrator, at the ab
address. The name and address
two referees will be required.

Closing Date — 28 Febru
1989.

Sandwell is a Non-Smok
Health Authority. (8411)A

PUBLIC HEALTH LABORATORY SERVICE
CENTRAL PUBLIC HEALT
LABORATORY

DIVISION OF MICROBIOLOGICA
REAGENTS AND QUALITY
CONTROL

SENIOR GRADE MICROBIOLOGIST

With experience in bacteriolo
and preferably with a high degr
or equivalent postgradua
research experience to join a sm
team working in the *Listeria* un
The job involves the developme
of identification and typi
methods for *Listeria monocyt
genes* and development of dia
nostic tests for listeriosis. Furth
details from Dr J McLaughlin tel
phone 01-200 4400 ext 3910/397

£11,921-£15,348 p.a. (inclusiv
(pay award pending).

Application form and job de
cription available from Personn
Department, Central Publ
Health Laboratory, 61 Colinda
Road, London NW9 5HT.

Tel. 01-200 4400 ext 3890.

Closing date 9th March 1989.

Please quote ref DMR/45.
(8402)A

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF HAEMATOLOGY

Applications are invited for a non-medical lectureship in the Department of Haematology which it is hoped to fill under the U.G.C. New Academic Appointments Scheme.

The Department has a major interest in the control of haematopoiesis and in its relevance to malignant haematology and bone marrow transplantation. It is hoped that applicants would develop their own interest in this area and, in liasion with clinical staff, provide scientific support for on-going studies.

Informal enquiries may be made to Professor J. C. Cawley or Dr. J. M. Davies, Tel. 051-709 0141 Ext 2811 or 2791.

Initial salary within the range £9,260 – £12,760 per annum on the Lecturer A scale.

The successful applicant will be appointed from 1st August 1989, or as soon as possible thereafter. Whilst there is no formal age limit, in accordance with the terms of the initiative to bring younger staff into the profession, it is likely that preference will be given to candidates at an early stage in their career.

Applications, together with the names of three referees, should be received not later than 10th March 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool. L69 3BX, from whom further particulars may be obtained.**

Quote ref. RV/254/N

An Equal Opportunity Employer (8385)A

WASHINGTON UNIVERSITY
SCHOOL OF MEDICINE
ST. LOUIS, MISSOURI
DEPARTMENT OF PHARMACOLOGY
CHAIRPERSON

Applications and nominations are invited for the position of Chairperson of the Department of Pharmacology. Candidates should be distinguished as scholars and scientists with demonstrated commitment to medical and graduate education.

Curriculum vitae and bibliography may be submitted to:

M.K. King, M.D.
Dean
Washington University School of Medicine
660 South Euclid — Box 8106
St. Louis, Missouri 63110

An Equal Opportunity/Affirmative Action Employer.
(NW3309)A

STUDENTSHIPS & ASSISTANTSHIPS

UNIVERSITY OF LONDON
St. Bartholomew's Hospital Medical College,
Department of Biochemistry

POSTDOCTORAL MOLECULAR BIOLOGIST
and
PhD STUDENT IN ENZYMOLOGY

SERC Postdoctoral Research Assistantship and Earmarked Research Studentship in Protein Engineering and Enzyme Mechanism. These two positions are available for 3 years from October 1989 for work on fundamental aspects of enzyme mechanism and molecular recognition, using the cysteine proteinase family of enzymes and mutants produced by site-directed mutagenesis. Applicants for the Postdoctoral position (£12,110-£13,330 p.a. inclusive of LW) should have experience or interest in molecular biology and those for the Studentship interest in structure-reactivity relationships and a good degree in Biochemistry or Chemistry.

Applications with CV and the names of two referees to:
Professor K. Brocklehurst or Dr. I. Phillips, Dept. of Biochemistry, St. Bartholomew's Hospital Medical College, University of London, Charterhouse Square, London EC1M 6BQ as soon as possible. (8410)A

UNIVERSITY OF OXFORD

INORGANIC CHEMISTRY LABORATORY
in association with St. Hilda's College or St. Catherine's College.

UNIVERSITY LECTURERSHIP IN INORGANIC CHEMISTRY

Applications are invited for a University lecturership in Inorganic Chemistry tenable with effect from 1 October 1989. Applicants may work in any branch of inorganic chemistry, including its borders with surface science, solid-state science, and biology. The successful candidate may be offered a tutorial fellowship by St. Hilda's College or St. Catherine's College on the terms described in the further particulars. Separate application need not be made for the college appointment. It is hoped to fund this appointment from money made available by the UGC under its new academic appointments scheme (NAAS). Under the conditions of the scheme the successful candidate will normally be near the start of an academic career and under 35.

Further particulars of the lecturership and of the college fellowships may be obtained from **Professor M. L. H. Green, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QH,** to whom a *curriculum vitae* and the names of three referees should be submitted by 28 February 1989.

UNIVERSITY LECTURERSHIP IN PLANT SCIENCE
in association with Pembroke College

Applications are invited for the above post to be filled from 1 October 1989. Preference will be given to candidates with an interest in Plant Biochemistry/Molecular Biology. Stipend according to age on the scale £9,260 to £20,615 per annum. The post may be held jointly with a stipendiary tutorial fellowship at Pembroke College. It is hoped to fund this appointment from money made available by the UGC under its new academic appointments scheme (NAAS): under the conditions of the scheme the successful candidate will be near the start of an academic career and under 35.

Applications (8 copies, or one from overseas candidates) should be sent by 3 March 1989 to **Professor F. R. Whatley, FRS, Department of Plant Sciences, South Parks Road, Oxford, OX1 3RA,** from whom further particulars may be obtained.

OXFORD CENTRE FOR MOLECULAR SCIENCES
POSTDOCTORAL RESEARCH ASSISTANT
PROTEIN CRYSTALLOGRAPHY

Applications are invited for the post of protein crystallographer whose general role will be to provide support for the collaborative crystallographic research programme of the Centre of Molecular Sciences based in the Laboratory of Molecular Biophysics under the direction of Sir David Phillips. The person appointed will have major responsibility for selection implementation and running of a new data collection facility (e.g. electronic area detector or image plates). The position allows scope for the development of independent research. Several years postdoctoral experience is required.

The post is funded until 30 September 1994 and is available immediately on the scale RAII (£14,500 – £19,310). If no suitable applicant is forthcoming, an appointment on the scale RA1A (£9865 – £15,720) may be considered, with an appropriate adjustment of duties.

Applications in writing with full CV and 2 referees should be made to **The Administrator, Oxford Centre for Molecular Sciences, c/o Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY,** from whom further details of the post can be obtained.

Closing date: 3 March 1989. (8420)A

The University is an Equal Opportunity Employer

POSTDOCTORAL POSITIONS —

analysis, identification and biocatalysis of fermentation byproducts

Three postdoctoral positions are available immediately to study byproducts obtained from fermentations. One position will be concerned with analysis and identification of fermentation byproducts; experience in separation of organic and inorganic compounds by all forms of chromatography including ion exchange, adsorption, electrophoresis, GC, HPLC, as well as other physical separation methods including distillation, lyophilization, liquid:liquid partitioning essential; must be familiar with spectral methods of analysis. Two positions will investigate the application of biocatalysis to compounds commonly found in fermentation wastes; experience will require a combination of skills including cultivation of microorganisms, fermentations, microbial transformations, and applied enzymology in the synthesis of organic chemicals; applications must have demonstrated expertise in metabolite production. All applicants must have demonstrated expertise in isolation (HPLC, TLC, GC), and in the identification of structures using NMR (carbon, proton), mass spectrometry, UV, IR.

Send resume and two letters of reference to **Professor Rex Montgomery, 200G HBRF, College of Medicine, University of Iowa, Iowa City, IA 52242. (319) 335-8067.** The University of Iowa is an equal opportunity, Affirmative Action Employer. Women and minorities are encouraged to apply. (NW3334)A

UNIVERSITY OF CAMBRIDGE

University Assistant Lecturers

in the Department of Physics

Several University Assistant Lecturers to take up appointment by 1st October 1989, or as soon as possible thereafter. Applicants will be expected to work in any aspect of physics but some preference will be given to those with interests in the main fields where the department is already active *viz* experimental and theoretical condensed matter physics, radio and millimetre wave astronomy, experimental and theoretical high energy physics.

A University Assistant Lecturer is appointed for three years, with the possibility of reappointment for a further two years. The statutory limit of tenure of a University Assistant Lecturer is five years, but all holders of the office of University Lecturer are considered for possible appointment to the office of University Lecturer during the course of their tenure.

The pensionable scale of stipends for a University Assistant Lecturer, not ordinarily resident in College, is £10,460 rising by seven annual increments to £14,500.

Further information may be obtained from the **Secretary of the Appointments Committee for the Faculty of Physics and Chemistry, Institute of Astronomy, Madingley Road, Cambridge, CB3 0HA**, to whom applications (ten copies), including a *curriculum vitae* and the names of three referees, should be sent so as to reach him not later than 31st March 1989. (8413)A

UNIVERSITY OF LIVERPOOL

Department of Geography

Applications are invited for two Lectureships in Physical Geography which it is hoped to fill under the U.G.C. New Academic Appointments Scheme. The Department is anxious to attract applicants with outstanding research achievements and promise. Preference will be given to candidates with research and teaching interests in one or more of the following fields: Remote Sensing; Coastal Geomorphology; Surface Processes (especially Soil and Water Chemistry). Informal enquiries may be made to Professor F. Oldfield, Tel. 051-794 2838.

Initial salary within the range £9,260 – £11,680 per annum on the Lecturer A scale.

Successful applicants will be appointed from 1st August 1989, or as soon as possible thereafter. Whilst there is no formal age limit, in accordance with the terms of the intitiative to bring younger staff into the profession, it is likely that preference will be given to candidates at an early stage in their careers.

Applications, together with the names of three referees, should be received not later than 10 March 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool. L69 3BX, from whom further particulars may be obtained. Quote ref. RV/264/N**

An Equal Opportunity Employer (8384)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL (University of London)

DEPARTMENTS OF PSYCHIATRY AND BIOCHEMISTRY

GRADUATE RESEARCH ASSISTANT

required to work on a study of Gene Expression in Schizophrenia. Previous experience in molecular biology or histology would be an advantage but not essential. Salary on scale £8,675-£11,680 per annum plus £1,650 per annum London Allowance.

Further details can be obtained from Dr. J. de Belleroche (01-846 7052) and applications with a full curriculum vitae together with the names and addresses of two referees to be sent to **The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St. Dunstan's Road, London W6 8RP,** to be submitted within three weeks of the appearance of this advertisement. (Quote Ref: 89/16.)

(8437)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF PATHOLOGY

RESEARCH ASSISTANT (Immunology) — Basic Grade or MLSO

The Division of Pathology has a substantial programme of research in AIDS and the immunodeficiency viruses supported by the MRC and the PHLS. The virological segment of a project to establish primate models is already well advanced. The models will be used for studies of the immune response following infection and for assessing vaccines and therapies. This post will provide support for a senior immunologist monitoring and analysing immune function and specific cellular responses following infection or vaccination. The work will involve handling tissues putatively infected with simian and human pathogens under category III containment conditions; analytical flow cytometry is available.

Applicants should ideally have experience in some areas of cellular immunology with an understanding of basic immunology and host responses to viruses. Experience in lymphocyte culture techniques and/or phenotyping is desirable but training can be given in those areas as necessary.

Applicants should possess an honours degree or a recognised technical qualification.

The post is funded by the MRC (AIDS-directed programme) for a fixed term of approximately 3 years.

Salary scale £6,993–£10,496 per annum.

National Health terms and conditions apply.

For further information contact Dr L A E Ashworth tel: Idmiston (0980) 610391

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG

Tel: (0980) 610391, to whom completed forms should be returned by 3rd March 1989 quoting Post No 1133. (8415)A

THE OPEN UNIVERSIT' FACULTY OF SCIENCE

RESEARCH FELLOW: PHYSICS

Applications are invited for post of Research Fellow within Physics Department, to work o project concerned with the me urement of directional emittan The successful candidate develop and use equipment measure the emittence of a vari of materials with accurac approaching those of standa laboratories, and will also co pare the measurements with ex ing theories of material surfac Among the materials to be inve gated are: coated glasses (of p ticular interest to the Europ glass industry); materials to act standards in the middle infra r materials for infra red instrum tation; and minerals of interest planetary science, includ remote sensing.

The work is particularly suita for someone who would enj working with considerable au nomy in a small research group.

Candidates should have a go honours degree in physics, appli physics or in a closely related ar of engineering or technology, a a Ph.D. degree (or have recen submitted a Ph.D. thesis) in exp imental science or technolo particularly in one of the followi areas: optics; radiometry; mid infra red spectrometry; emis metry.

The post is available for t years from 1st October 19 Starting salary will be on the scale for Research Staff: £9865 £15720 at a starting point up £11070 per annum (at prese rates).

Application forms can obtained from Mrs. B. Wood (8044/2), Faculty of Science, Wi pey II, The Open University, W. ton Hall, Milton Keynes, MI 6AA or telephone Milton Keyn (0908) 653993: there is a 24-ho answering service on 653868.

Closing date for applicatior 6th March.

Equal Opportunity is Universi Policy (8395)A

UNIVERSITY OF BIRMINGHAM
SPACEFLIGHT INSTRUMENTATION FOR THE SPECTRUM-X MISSION (Ref PH2015)

A SERC-funded research vacancy exists in the School of Physics and Space Research for either a postgraduate Research Associate or a postdoctoral Research Fellow. The successful candidate will work on the development of the flight hardware and/or the on-board software for an X-ray telescope to be flown on the Soviet SPECTRUM-X mission in 1993. Applications are invited from honours graduates, or persons who have recently obtained a higher degree, in Physics or a relevant engineering discipline. It is anticipated that the successful applicant will have some experience in the development of microprocessor systems. The post is to be filled as soon as possible and is tenable for 3 years. The starting salary will be on the Research Associate 1B scale, £8,675–£11,680, or on the first four points of the Research Fellow 1A scale, £9,865–£15,720, as appropriate.

For further particulars of the above post and an application form, telephone 021-414 6383 quoting reference number PH2015. Applications (3 copies) should be sent to the **Senior Assistant Registrar, Faculty of Science, PO Box 363, Birminham B15 2TT** by 3 March 1989.

An Equal Opportunities employer

(8434)A

GEOCHEM GROUP LIMITED, CHESTER STREET, SALTNEY, CHESTER CH4 8RD
TEL: 0244 671121
ISOTOPE SPECIALIST

Applications are invited for a position in our rapidly expanding mass spectrometry department based at our laboratories in Chester.

The professional we are seeking will have proven experience in the measurement of the stable isotopes of Carbon, Sulphur, Oxygen, Hydrogen and Nitrogen. Knowledge of the relevant preparation techniques is essential.

Reporting to the manager of Mass Spectrometry, the successful applicant will be responsible not only for the routine operations of our busy isotope laboratory but will be expected to develop Geochem's isotope service. In addition the post carries a significant involvement in research and development.

An attractive salary (negotiable according to age and experience) is offered, together with a comprehensive package of benefits. Applicants should write, enclosing a detailed C.V., to **Gareth Harriman, Mass Spectrometry Manager** by no later than 6th March. (8442)A

UNIVERSITY OF LIVERPOOL
Department of Genetics and Microbiology
Senior Research Assistants/Research Associates

Two positions are immediately available in a laboratory using the filamentous fungus *Aspergillus nidulans* to study aspects of eukaryotic DNA repair and control of the cell cycle.

Position 1 is a three year post, funded by the Wellcome Trust, to clone, sequence and further characterize genes involved in the repair of alkylation damage in DNA.

Position 2 is initially a one year post, extendable for a further two years, using recombinant DNA techniques to assess the feasiblity of identifying human DNA repair and cell cycle genes by complementation in *Aspergillus nidulans*.

Applicants for both posts should have, or expect to gain shortly, a Ph.D. in a relevant area of research.

Initial salary for both posts will be within the range £9,865 – £13,365 per annum.

Informal enquiries should be made to Dr. P. Strike on 051-794 3620.

Applications, together with the names of three referees, should be received not later than 3rd March 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool. L69 3BX, from whom further particulars may be obtained. Quote ref. RV/254/N**

An Equal Opportunity Employer (8387)A

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serc **SEPARATIONS INITIATIVE**

CO-ORDINATOR FOR BIOSEPARATIONS RESEARCH

The SERC Separation Processes Initiative is a major managed programme of research into separation and purification technology. This includes research into selective absorption, membrane separations, highly selective separations and mechanical methods.

The role of the Co-ordinator (for the biotechnological aspects of the programme) will be to stimulate research proposals and co-ordinate the activities of the research groups in the programme. This will involve liaison with the Membranes Co-ordinator and the provision of expert advice on biotechnology to the Programme Manager. Candidates should have experience of downstream processing in the biotechnology field, and those proposing secondment from an academic institution or industry are especially invited.

Please send full CV and covering letter to:
Neil Viner,
The Biotechnology Directorate,
Science and Engineering Research Council,
Polaris House, North Star Avenue, Swindon SN2 1ET
Telephone: (0793) 411279
Closing date for applications 20/3/89

This part-time, fixed term appointment will be for 2 years in the first instance with possible extension, and location by arrangement, although considerable travel will be required. Salary is negotiable (8452)A

ENDOWED PROFESSORSHIP IN MOLECULAR GENETICS

The University of Cincinnati and the State of Ohio have created an endowed professorship in the area of Molecular Genetics. This professorship, named the Ohio Eminent Scholar, is designed to add an outstanding scientist to the newly formed Department of Molecular Genetics, Biochemistry and Microbiology in the College of Medicine. Areas of interest include immunology, differentiation and development and human genetics. The department has major research programs including the recent award of a Program of Excellence in Molecular Biology from the Heart, Lung and Blood Institute of NIH. The Eminent Scholar will fulfill a pivotal role in expanding strength and interactions in the College. The University of Cincinnati is a comprehensive university in the state system. Curriculum vitae should be sent to:

Dr. Jerry B Lingrel
Department of Molecular Genetics, Biochemistry & Microbiology
University of Cincinnati
College of Medicine
Cincinnati, Ohio 45267-0524

Affirmative Action/Equal Opportunity Employer (NW3317)A

RESEARCH POSITIONS
IN MOLECULAR/CELL BIOLOGY

Post-doctoral or Assistant professor level positions are available immediately in the Department of Cell Biology. The person(s) appointed will be member of an English-speaking research group investigating the role of oncogenes and their interactions with carcinogens in normal and HBV-infected hepatocytes in culture and in transgenic mice, and on mechanisms underlying the control of hepatocyte-specific gene expression.

Applicants with an excellent background in general biochemistry and molecular/cell biology should have experience in one of the following areas: general cell culture techniques; recombinant DNA techniques; virology. Salary is negotiable and will be based upon the candidate's previous training and experience (BATIIa/BATIb). Initial appointments will be made for 2 years with possibilities for extension for additional 2 years or for permanent appointments. Send CV and names of at least 2 referees before March 15 1989 to: **Prof. Dr. Dieter Paul, Department of Cell Biology, Fraunhofer Institute of Toxicology, 3000 HANNOVER 61, Nicolai-Fuchsstrasse 1, Federal Republic of Germany.**

(W5842)A

LONDON BIOTECHNOLOGY LTD

Molecular Biologist/Enzymologist required immediately to work on 2-year contract involving site-directed mutagenesis of an enzyme. Candidates must have laboratory experience of (1) gene cloning and other relevant molecular biology techniques, (2) enzyme assays and kinetic analysis. Qualifications: minimum of MSc in relevant area. Salary £8675 (year 1) and £9260 (year 2) both plus £1450 L.A. Send application and full c.v. to **Dr M R Hollaway, Biochemistry Dept, University College London, Gower Street, London WC1E 6BT. or telephone 01-387 7050 xt. 7033.** *Equal Opportunities Employer.*

(8436)A

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF CHEMISTRY

Applications are invited for the newly established LECTURESHIP to be filled from a date to be arranged. The successful candidate will be expected to work closely with the incoming Professor of Physical Chemistry, Dr A Hamnett and should, therefore, have an interest in Modern Electrochemical and Spectroscopic Techniques.

Salary will be at an appropriate point on either Lecturer Grade A £9,260-£14,500 p.a. or Grade B £15,105-£19,310 p.a. according to qualifications and experience.

Further particulars may be obtained from the **Senior Assistant Registrar (Establishments), The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU,** with whom applications (three copies), with the names and addresses of 3 referees, should be lodged not later than 22nd March 1989. (8439)A

FIELD MUSEUM OF NATURAL HISTORY
Paleontologist

Applications are invited for a career-track position in paleontology, preferably at the level of assistant curator. Specimen-oriented research is required in invertebrate or vertebrate paleontology, paleobotany, or related fields. Our principal criteria are research excellence and an ability to interact with staff in other areas of paleontology. Responsibilities also include curation of relevant collections, limited participation in exhibition and education programs, and some administrative work.

The Department of Geology has 8 curatorial positions (7 in paleobiology) and 6 supporting positions. The department maintains extensive collections in nearly all areas of paleontology, and actively participates in the programs of universities in the Chicago area. Applicants should have a Ph.D. and should mail a curriculum vitae, description of current and future research program, and names of three references (with phone number, if possible) to: **Search Committee, Dept. of Geology, Field Museum of Natural History, Chicago, IL 60605,** by March 30, 1989. *An Equal Opportunity Employer.* (NW3318)A

PUBLIC HEALTH LABORATORY SERVICE BOARD
PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

PATHOLOGY SECTION
LEGIONELLA SECTION

BASIC/SENIOR GRADE MICROBIOLOGIST

A graduate or postgraduate is required to investigate the growth of *Legionella pneumophila* in biofilms of complex aquatic communities generated using continuous culture techniques. The project is ongoing and the vacancy exists due to staff regrading. The position is immediately available for a current duration of at least 13 months and involves collaboration with a major water authority.

A microbiology or biological sciences degree is preferred and experience of aquatic/medical microbiology or continuous culture would be advantageous.

Salary scale: Basic Grade Microbiologist, £7,825-£10,496 per annum. Senior Grade Microbiologist, £11,164-£14,591 per annum.

NHS terms and conditions.

Further information may be obtained from Dr C.W. Keevil or Dr J.V. Lee tel. (0980) 610391.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Telephone (0980) 610391 ext 240, to whom completed forms should be returned by 3rd March 1989 quoting Post No 0627. (8399)A

Signal Transduction/ Cell-Cell Interactions

Post-doctoral position available to study chemotaxis and cell-cell contact. Projects include studies of the chemotactic receptor and its kinase (Meier and Klein, PNAS **85**, 2181, '88), GDP-modulated phosphorylation and relation to G protein (Anschutz, et al., PNAS, in press) and glycolipid anchoring of cohesion molecules (Sadeghi, et al., PNAS **85**, 5512, '88).

Send CV and addresses of three references to: **Dr. Claudette Klein, E.A. Doisy Dept. of Biochemistry, St. Louis Univ. Medical School, 1402 South Grand Blvd., St. Louis, MO 63104.**

EOE/APP (NW3321)A

THE AFRC INSTITUTE O HORTICULTURAL RESEARCH

Wellesbourne

TEMPORARY HIGHER SCIENTIFIC OFFICER

A plant breeder (HSO grade) required to direct a breeding pr gramme to produce improv varieties of lettuce for the UK a Europe. The breeding pr gramme, which is to be financ by the French Seed Compa Clause Semences Professionnel (CSP) for a period of up to 5 yea will be linked with a breeding pr gramme based at CSP's breedi station in the South of France, a will coordinate with strate MAFF funded research on lettu breeding carried out at IH Wellesbourne.

Qualifications: First or upp second class honours degree botany or genetics and a PhD or least 3 years' experience in pla breeding. Good knowledge statistical analysis and experime tal design will be expected, and t ability to speak French an advar age.

Salary: In Range £10,02 £13,460.

Further details and applicati form from the Personnel Offic Institute of Horticultural R search, Bradbourne House, E Malling, Maidstone, Kent ME 6BJ, quoting Ref 23/W. Closi date 9-3-89.

The AFRC is an Equal Opp tunities Employer. (8423)A

THE AFRC INSTITUTE OF HORTICULTURAL RESEARCH

Littlehampton West Sussex

SCIENTIFIC OFFICER

Short-term post (18 months)

A Scientific Officer is required f a short-term contract appointmer funded by the Agricultural Gen tics Company to investigate th use of insect-parasitic rhabditi nematodes (Steinernema an Heterorhabditis spp.) for the cor trol of mushroom phorid flie (Megaselia halterata).

Qualifications: First or upp second class honours degree appropriate subject.

Salary: In range £8,574-£10,994

Further details and applicatio form from the Personnel Officer Institute of Horticultural Re search, Bradbourne House, Eas Malling, Maidstone, Kent ME1 6BJ, quoting Ref. 31/L. Closin date 9-3-89.

The AFRC is an Equal Oppor tunities Employer. (8424)A

UNIVERSITY OF MANCHESTER

RESEARCH ASSOCIATE

Applications are invited for the above post in the Department of Chemistry to work on the crystallographic analyses of concanavalin-A and its interactions with saccharides. Experience in structure refinement would be useful. The position is available immediately for 36 months. Starting salary £10,460 p.a. Application forms from the **Registrar, The University, Manchester M13 9PL. (Tel: 061 275 2028)** Quote ref. 33/89/NA. Telephone enquiries to Professor J.R. Helliwell 061 275 4686.

The University is an equal opportunities employer. (8392)A

M O N A S H

U N I V E R S I T Y

DEPARTMENT OF MATHEMATICS

POSTDOCTORAL RESEARCH

Postdoctoral Research Fellow required for project modelling force and energy balance in solar magnetic structures. Experience in numerical modelling of physical systems and solution of PDE's essential. Knowledge of solar physics and magnetohydrodynamics preferred. Annually renewable appointment from April 1989 to December 1991, subject to funding. Starting salary $A26,617 p.a. Enquiries to Dr. P Cally, phone 61-3 565 4771.

Applications including Ref. no. 40163A, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Vic. 3168 Australia by 31/3/89.

AN EQUAL OPPORTUNITY EMPLOYER.

(W5890)A arm06836

POSTDOCTORAL/ RESEARCH ASSOCIATE POSITION

Available immediately for Ph.D. or M.D. to study the function of the hematopoietic extracellular matrix. Projects include matrix purification, the interaction of matrix with hematopoietic growth factors, and the effects of drugs on bone marrow function.

Please submit curriculum vitae, a brief description of research experience, and three letters of reference to: **Dr. Sharon Luikart, Department of Medicine, Box 325 University of Minnesota Hospitals and Clinics, Minneapolis, MN 55455.**

We are an Equal Opportunity Employer. (NW3315)A

POSTDOCTORAL RESEARCH POSITION IN ATMOSPHERIC CHEMISTRY

In the Biogeochemistry Department of the Max-Planck-Institute for Chemistry a research position is available in an interdisciplinary group studying the exchange of gasses between terrestrial ecosystems and the atmosphere.

The experimental approach includes plant physiology and micrometerological techniques to measure turbulent fluxes of gases above vegetated surfaces.

The applicant would be expected to have training in micrometerology and atmospheric chemistry. Experience in atmospheric trace gas analysis would be an advantage.

The institute offers outstanding research facilities and travel funds. The project is funded for three years with an initial 6 month probationary period. The position is available from March 1989. Salaries scale up to DM 58,000. Interested candidates should send C.V. and references to:

Prof. Dr. M. O. Andreae, Biogeochemistry Dept., Max-Planck-Institute for Chemistry, P.O. Box 3060, D-6500 Mainz, Federal Republic of Germany. (W5874)A

POSITION IN FLOW CYTOMETRY AND EXPERIMENTAL HAEMOPOIESIS RESEARCH

Leukaemia Research Fund Centre
Institute of Cancer Research, London

A position is available at the Chester Beatty Laboratories, Fulham Road, London, for an individual with experience in flow cytometry and cell sorting to join a group studying cellular and molecular aspects of haemopoietic stem cells. The person appointed will be responsible for a FACS 440 dual laser cell sorter and will participate fully in the research programme. The position could be suitable either for a post-doctoral scientist (salary range £13,130-15,950 p.a.) or a Senior Research Officer/Senior Technician (salary range £10,512-14,758 p.a.).

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Applications in duplicate, with CV and names and addresses of two referees, should be sent to the **Personnel Officer, The Institute of Cancer Research, 17a Onslow Gardens, London SW7 3AL** quoting reference 2.89.S.N. 70 (8393)A

The Food and Drug Administration

The Food and Drug Administration is recruiting for the Director, Division of Virology in the Center for Biologics Evaluation and Research located in Bethesda, Maryland. The position is in the Senior Executive Service with a salary range of $68,700 to $76,400. The Director is responsible for directing a staff of 55 in assuring the safety and effectiveness of biological products used in the prevention, treatment and mitigation of viral and rickettsial diseases. Applicants must have: executive level experience in managing broad scientific research or regulatory programs; demonstrated strong leadership and managerial abilities in directing complex scientific programs; ability to deal effectively with high level governmental officials, the scientific and academic communities, medical and health-related organizations, representatives of the regulated industry and others; and the ability to implement equal employment opportunity programs. Applicants should have an M.D. degree with board certification or a Ph.D. in a related scientific discipline with post doctoral training and a national/international reputation in viral and rickettsial biological products or related disciplines.

If you are interested and want to be considered, send a curriculum vitae to: **Jim Keady, Division of Human Resources Management, Food and Drug Administration, Parklawn Building, Room 4B-09, 5600 Fishers Lane, Rockville, Maryland 20857.**

If you need more information, call Jim, collect on (301-443-1755).

This position can also be filled by current Commissioned Officers of the U.S. Public Health Service, or by former officers willing to rejoin the Corps.

Applications will be accepted until March 31, 1989.

An Equal Opportunity Employer. (NW3329)A

FACULTY POSITION

Applications are invited for a tenure-track position in the Division of Dermatology at Washington University School of Medicine. Applicants should hold an M.D., Ph.D. degree with post-doctoral experience, and have completed an internship. It is expected that the applicant will have a demonstrable potential to develop an independent research program in areas of cellular and/or molecular biology. The successful candidate will also be expected to undertake clinical dermatology training with the ultimate goal of board certification and sharing the clinical teaching responsibilities on an equal basis with the existing faculty. New laboratory space, start-up support and the facilities of the department are available. Applicants should send curriculum vitae and a brief statement of research interests to: **Arthur Z. Eisen, M.D., Head, Division of Dermatology, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, Missouri 63110.** *An Equal Opportunity/Affirmative Action Employer.* (NW3324)A

ASSISTANT PROFESSOR

Tenure track position is available in the Department of Medical Microbiology and Immunology, Ohio State University College of Medicine, for either a retrovirologist or a viral immunologist interested in neurological disease. Opportunities to collaborate closely with other groups involved in viral immunology, molecular virology and neuroimmunology. The OSU Medical Center is one of the designated AIDS Clinical Trial Groups. Applicant should hold M.D. or Ph.D. degree and at least two years experience, and have a strong record of scholarly achievement. Will be expected to establish an active research program and participate in medical student and graduate teaching programs. Send curriculum vitae, a summary of research interests, and three letters of recommendation to: **Ronald Glaser, Ph.D., Department of Medical Microbiology and Immunology, The Ohio State University College of Medicine, 333 West 10th Avenue, Columbus, Ohio 43210.**

The Ohio State University of an Equal Opportunity/Affirmative Action Employer. (NW3331)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA

HIGHER SCIENTIFIC OFFICER (BAND I)
(21-month appointment)

TISSUE CULTURE AND CYTOLOGY DEPARTMENT

A plant scientist is required to explore the potential of asymmetric cell fusion in potato improvement. Knowledge of plant tissue culture techniques is required and experience of protoplast manipulation would be an advantage. A technical support worker is also available for this position.

Salary: £10,026–£13,460

Non-contributory superannuation.

Qualifications: First or upper second class honours degree in a related discipline with at least two years' relevant post-graduate research experience which may be a course of research and study leading to a Ph.D. degree.

The Institute is an equal opportunities employer.

Curriculum vitae complete with the names and addresses of three referees should be sent to **Mr I Paxton, Personnel Officer, by 3 March 1989** quoting the appropriate reference **(Ref. TCC/1/89)**. (8429)A

ASSISTANT/ASSOCIATE PROFESSOR POSITION, NEUROSCIENCE

Tenure track position in Department of Neuroscience available beginning 04/17/89. Primary teaching responsibilities will be Medical and Dental neuroanatomy and cellular/systems neuro-funded research program on the neurotoxicology of drugs of abuse with emphasis on neurophysiological/neuropharmacological techniques. Collaboration with other established laboratories investigating these issues is expected. Application, including curriculum vitae and the names of three references should be sent by 04/03/89 to **Don W. Walker, Ph.D., Department of Neuroscience, Box J-244, J.H.M.H.C., College of Medicine, University of Florida, Gainesville, FL 32610–0244.**

The University of Florida is an Equal Opportunity/Affirmative Action Employer

(NW3328)A

RESEARCH CHEMIST

To conduct the preparation of carcinogen DNA/RNA adducts as model reference compounds for chromatography characterization and spectral measurements utilizing knowledge in advanced organic, analytical and chromatographic sciences for use in the identification and structural elucidation of adducts resulting from the interaction of polycyclic aromatic hydrocarbons with DNA/RNA in vivo using fish as the model and in vitro studies. Chromatographic separation and study of optical isomers of a proprietary drug and separation of deoxynucleosides and penta, hexa, and octa derivatives of an ulcer drug. Salary: $23,750.00 per year/40 hour week. Requirements: Masters level degree with an emphasis in analytical chemistry. Applicant must submit evidence of one graduate project in organic chemistry and two or three letters of reference as to research abilities. Applications to: **John Scott, E.S. Supervisor, Job Service, P.O. Box 59, Jefferson City, Missouri 65104.** Refer to Job Order Number 238813. (NW3322)A

HUMBOLDT STATE UNIVERSITY

Lecturer Equivalent to Assitant Professor Community Ecology/ Conservation Biology

One-year temporary position. Ph.D. is required and university teaching experience is preferred. Candidates must have expertise in community ecology and/or conservation biology. Demonstrated interest in wildlife management and in manipulated populations preferred. Candidates with a strong background in mathematics, professional wildlife experience, and/or with an understanding of consumptive use of wildlife will be given preference. Demonstrated research experience in the appropriate specialties is essential. Applicants should submit a curriculum vitae (including reprints), transcripts of academic work, and three letters of reference to **Dr. Richard L. Ridenhour, Dean, College of Natural Resources, Humboldt State University, Aroata, CA 95521. Phone (707) 826-3561.** Closing date is 30 March 1989. *Humboldt State University is an Equal Opportunity/Affirmative Action Title IX Employer.* (NW3320)A

UNIVERSITY OF CANTERBURY
New Zealand

LECTURER IN MICROBIOLOGY
DEPARTMENT OF PLANT AND MICROBIAL SCIENCES

Applications are invited for the above position. Applicants should have a Ph.D. degree in Microbiology. Preference will be given to a candidate with research experience in non-medical microbiology and microbial metabolism. The appointee will be expected to contribute to the teaching of microbiology courses at all levels and first-year biochemistry, and to pursue an active research programme including the supervision of M.Sc. and Ph.D. students.

The salary for Lecturers is on a scale from NZ$35,000 to NZ42,500 (bar), NZ$44,000 to NZ$46,000 per annum.

Further particulars and Conditions of Appointment may be obtained from Appointments (36015), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Applications close with A.W. Hayward, Registrar, University of Canterbury, Private Bag, Christchurch, New Zealand, on 28 April 1989. (W5883)A

POSTDOCTORAL POSITION

available for membrane transport studies and studies on structure-function analysis of a membrane transport protein. Requires expertise in protein biochemistry and molecular biology. Salary negotiable, commensurate with experience. Send letter of application, curriculum vitae and two references to: **Dr. Ada Elgavish, Department of Pharmacology, University of Alabama School of Medicine, Birmingham, Al 35294.** An Equal Opportunity/Affirmative Action Employer.

(NW3326)A

THE UNIVERSITY OF QUEENSLAND
Brisbane, Australia

RESEARCH ASSOCIATE GEOLOGY
Department of Geology an Mineralogy

Ph.D graduate in geology to j and coordinate, a research t working on the structural, m morphic, sedimentologic, and tonic analysis of terranes in par the New England Fold Belt southeast Queensland, Austra A background in any of the ab disciplines is required and exp ence in structural geology and tonic analysis is preferred. position is funded by the Aus lian Research Council and is pected to last for up to three ye

The work will involve consic able field mapping, particularl the early years, and interact with other individuals and gro working on associated projects

Salary: A$26,617 per annu Closing date: 31 March 19 Reference No: 07189. Assista with travel provided.

Further information may obtained from Dr R.J. Holcom Department of Geology Mineralogy, St Lucia, Quee land, 4067, Australia, to wh applications should be directed

Equal Opportunity in Empl ment is University Policy.

(W5887)A

RESEARCH TECHNICIAN

needed to maintain plant cell cu tures and isolate plant cell walls Cell wall polysaccharides an oligosaccharides will be solu bilized, purified, and structurall characterized using enzymat and chemical methods. Exper ience in these techniques desir able. B.S. required/M.S. preferre in biology or biochemistry. Salar negotiable depending on exper ience. Send applications by Apri 1, 1989 to **Dr. Alan Darvill or Dr Peter Albersheim, Co-Directors Complex Carbohydrate Researc Center, University of Georgia P.O. Box 5677, Russell Researc Center, Athens, GA 30613 USA**

An EEO/AA institution.

(NW3333)A

School for Field Studies

School for Field Studies seeks Sr. & Assoc. faculty for summer programs; Ethnobot./Ecuador; Landscape Ecol./Colorado; Tropical Ecol./Costa Rica; Acid Rain/Adirondacks; Wildlife Mngt./Kenya; Cult. Ecol./Himalayas; Bald Eagles/Alaska; Coral Reef Ecol. & Marine Pks. Mngt./Virgin Isles; Monkseals/Greece; Spinner Dolphins/Hawaii; Humpback Whale Ecol./N. Atlantic; Dolphins/Alabama. Ph.D. (MS. for Assoc. Fac.), excel. teaching skills, field resch. exp., enthus. for experiential ed. Send detailed letter, cv and refs. to the **Dean, SFS, 16 Broadway, Beverly, MA 01915 (508) 927-7777.** (NW3203)A

RESEARCH SCIENTIST (ASSISTANT PROFESSOR)

The Department of Medical Microbiology and Immunology, Ohio State University Medical Center, is seeking a person to work collaboratively with a research group in the area of central nervous system/immune system interactions. Applicant should hold an M.D. or Ph.D. degree with experience in an area concerned with neuroimmune interactions. Salary commensurate with experience. Submit curriculum vitae, including reprints, and three letters of recommendation to: **Ronald Glaser, Ph.D., Department of Medical Microbiology and Immunology, The Ohio State University College of Medicine, 333 West 10th Avenue, Columbus, Ohio 43210.**

The Ohio State University is an Equal Opportunity/Affirmative Action Employer. (NW3332)A

THE HANNAH RESEARCH INSTITUTE

IMMUNOLOGIST/ BIOCHEMIST

An immunologist or a biochemist with experience in immunology is required to investigate the autocrine control of milk secretion. The appointee will join a team within the Director's Research Group which is studying local mechanisms regulating mammary function. Techniques will include PLC, polyclonal and monoclonal antibody production, and radioimmunoassay and enzyme immunoassay development. The post is funded for 2 years.

Applicants should possess a first or upper second class degree in immunology, biochemistry or a related discipline, with at least 2 year's relevant experience.

Appointment will be at Higher Scientific Officer grade, with starting salary up to £10,994. A non-contributory superannuation scheme is operating.

Further details may be obtained from The Secretary, The Hannah Research Institute, AYR KA6 HL to whom applications, including the names of two referees should be forwarded by 3 March 1989 quoting reference HRI/165. (8453)A

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Postdoctoral Associate

To apply molecular cell biological methods to study of lipid movement and lipid droplet interaction with membranes in differentiated mammary epithelial cells. Position available from 1 April, 1989. Initial appointment for one year with possible renewal for three additional years. Send CV and names, addresses and telephone numbers of three references to:

T. W. Keenan, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061 USA.

An equal opportunity/affirmative action employer. (NW3330)A

POSTDOCTORAL POSITION

available to study genetic defects resulting in enzyme deficiencies in UMP synthase and possible immune system defects associated with hereditary orotic aciduria. Other research involves analysis of the bifunctional domain structure of UMP synthase using site-directed mutagenesis and vector expression in bacterial and cell culture systems. Applicants with a strong background in molecular biology and/or protein biochemistry are preferred. Two to 3 years of funding is available starting at $22,000. Send curriculum vitae and names of three references to: **Dr. D. Parker Suttle, Department of Pharmacology, St. Jude Children's Research Hospital, 322 North Lauderdale, Memphis, TN 38101–0318.**

An Equal Opportunity/Affirmative Action Employer. (NW3323)A

Tokyo Institute for Immunopharmacology, Inc.

Junior and Senior Molecular Biologists/ Immunologists

The Institute is seeking molecular biologists with a strong background in mammalian genetic engineering, and is especially interested in candidates with experience in the construction and screening of genomic and cDNA library, gene expression in mammalian cells and/or establishment of transgenic mice. We also invite applications from cellular immunologists who have strong interest in macrophage development, and activation.

She/he is expected to have a Ph.D. with postdoctoral training or equivalent for senior positions, or a Ph.D. with lesser training for junior positions. The appointees will join an active research group involved in the study of molecular immunology, focusing on molecular mechanisms of macrophage development and activation.

Applicants should submit curriculum vitae, list of publications, description of research interests and letters of reference to:

Takeshi Yoshida, M.D., D. Med. Sci., Director, Tokyo Institute for Immunopharmacology, Inc., 3-41-8 Takada, Toshima-ku, Tokyo 171, Japan. (W5886)A

HUMBOLDT STATE UNIVERSITY

LECTURER EQUIVALENT TO ASSISTANT PROFESSOR COMMUNITY ECOLOGY/ CONSERVATION BIOLOGY

One-year temporary position. Ph.D. is required and university teaching experience is preferred. Candidates must have expertise in community ecology and/or conservation biology. Demonstrated interest in wildlife management and in manipulated populations preferred. Candidates with a strong background in mathematics, professional wildlife experience, and/or with an understanding of consumptive use of wildlife will be given preference. Demonstrated research experience in the appropriate specialties is essential.

Applicants should submit a curriculum vita (including reprints), transcripts of academic work, and three letters of reference to Dr. Richard L. Ridenhour, Dean, College of Natural Resources, Humboldt State University, Arcata, CA 95521. Phone (707) 826–3561. Closing date is 30 March 1989.

Humboldt State University is an Equal Opportunity/Affirmative Action Title IX Employer. (NW3335)A

AWARDS

UNIVERSITY OF BRISTOL Zoology Department

POST-GRADUATE AWARDS 1989

Applications are invited from new or prospective Graduates in Zoology or a related discipline for places in the following research areas:

Adaptive physiology of visual pigments. (Dr J.N. Lythgoe)

Rhythm of foraging activity in marine grazing molluscs. (Dr C. Little)

Neuropeptide function/action in neural cell cultures. (Dr L.W. Haynes)

Helicoidal ultrastructure of outer casings in cryptobiotic animals. (Dr A.C. Neville)

Photoperiodic control of seasonal breeding in birds. (Professor B.K. Follett)

Neurobiology of behaviour in amphibian embryos. (Dr A. Roberts)

Ecology and social behaviour of mammals. (Dr S. Harris)

Biochemical defences of marine organisms. (Dr T.E. Thompson)

Places funded by SERC will be available for suitable applicants. Applications to **Dr T.E. Thompson, Zoology Department, University of Bristol BS8 1UG.** (8455)N

continued on page 2

INSTITUTE OF CHILD HEALTH
University of London

PhD STUDENTSHIPS
(Minimum value £5900 p.a.)

Applications are invited for three newly-established PhD studentships to work in the following fields of research in the departments of Immunology (2) and Paediatric Genetics (1).

Cytokine regulation of immunoglobulin isotype switching and secretion in man. (Dr R Callard)

Molecular Genetics of Anglemans syndrome: a chromosomal syndrome associated with the deletions of chromosome 15. (Professor M E Pembrey and Dr S Malcolm)

The immunoregulatory function of antigen in the gastrointestional tract. (Dr M Turner and Dr S Strobel)

Both departments have a wide range of current research interests and have excellent laboratory facilities.

Candidates should normally be not more than 25 years of age on 1st October 1989 and have been ordinarily resident in the UK for a minimum period of 3 years, and either possess, or hope to obtain, a First or Upper Second Class Honours Degree in an appropriate subject.

Further information can be obtained from **The Registrar, The Institute of Child Health, 30 Guilford St, London WC1N 1EH. Telephone 01-242 9789 Ext: 2679.**

Applications in the form of a Curriculum Vitae and the names of two academic references should be submitted as soon as possible. (8445)F

University College London
The Galton Laboratory
Department of Genetics and Biometry

Ph.D. Studentship

Funded by the Tuberous Sclerosis Association of Great Britain, this studentship is available in the laboratory of Dr. Jonathan Wolfe. The student will attempt to clone the chromosome 9 linked gene for the disease tuberous sclerosis by 'reverse genetics'. This will involve making genomic and linking libraries from appropriate somatic cell hybrids, constructing long range physical maps of the relevant region by pulse field gel electrophoresis and isolating candidate genes.

Applicants should have, or expect to have, a first or upper second class honours degree in an appropriate subject.

Further information can be obtained from **Dr. J. Wolfe, The Galton Laboratory, 4 Stephenson Way, London NW1 2HE (01-387 7050) X 5076)** to whom applications including a curriculum vitae and the names and addresses of two referees should be sent by March 31st 1989.

Equal opportunities Employer. (8435)F

SCHOOL OF PHARMACY
UNIVERSITY OF LONDON
Department of Pharmaceutical Chemistry
POSTGRADUATE STUDENTSHIPS

A number of postgraduate studentships funded by SERC, Charities and private sources will be available in this Department for postgraduate studies commencing July-October 1989.

Remuneration depends on the source of funding and on age and experience and will be in the range at current rates of £3,630-£5,115 pa. Applicants for training in any branch of Pharmacy, Chemistry, Biochemistry and related subjects will be eligible.

Research areas include drug design, synthesis, spectroscopy, molecular graphics, pharmaceutical analysis, receptor and enzyme structure and function, immunochemistry, molecular parasitology, lipid biochemistry and neurochemistry.

Applicants should write directly to **Professor W A Gibbons, Head of Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX.** (8449)F

THE INSTITUTE OF CANCER RESEARCH: ROYAL CANCER HOSPITAL
(UNIVERSITY OF LONDON)

A number of POSTGRADUATE RESEARCH STUDENTSHIPS, tenable for up to three years leading to the PhD degree of the University of London, will be available at the Institute of Cancer Research from 1 October 1989. The Institute is based on two sites, at Sutton, Surrey and in Chelsea, London, SW3, and its research work is carried out in close collaboration with the Royal Marsden Hospital. Projects offered for 1989 are:—

Cell and Molecular Biology: Structural and functional analysis of a transcription protein which binds to enhancers and promoters of erythroid-expressed genes (G. A. Goodwin).
Mechanisms of ras oncogene transformation (C. J. Marshall).
The role of cloned transcription factors in mammalian development and sex determination. (A. Ashworth).

Pathology: Structure and function of myoepithelial cells (B. A. Gusterson and P. Monaghan)
Molecular and biological analysis of human c-erbB-2 (B. A. Gusterson).

Radiotherapy Research Unit: The sensitivity of human tumour cells to radiation: underlying molecular mechanisms (G. G. Steel and T. J. McMillan).

CRC Biomolecular Structure Unit: Synthesis and evaluation of DNA sequence-specific compounds as potential anti-cancer agents (T. C. Jenkins).

CRC Laboratory, Drug Development: Synthesis of some S-adenosyl-L-homocysteine derivatives and their nucleoside precursors (M. Jarman and P. Serafinowski).
The synthesis of inhibitors of steroid biosynthesis for the treatment of hormone-dependent cancers.

Medicine, Experimental Unit: Generation of immune response to tumours using antibodies that bear an internal image of specific tumour antigenes (C. J. Dean).

Physics: Modelling of tumour physiology using Positron Emission Tomography (M. A. Flower and S. Webb).
Dose optimisation for conformation radiotherapy (S. Webb and W. Swindell).
Localisation, quantitation and editing in human and animal proton NMR spectroscopy (M. O. Leach).
The application of silicon radiation detectors to beta and gamma ray imaging (R. J. Ott).
Quantitative ultrasound imaging of tumour vasculature (J. C. Bamber and D. O. Cosgrove).
Perceptual assessment of medical images (J. C. Bamber and S. Webb).

Studentship stipends will be at the normal Research Council rates, plus a supplementation of £1000 pa. Candidates should have or expect to gain a I or II(i) Honours degree or a Master's degree. Further details are available in the postgraduate prospectus, which is obtainable, together with an application form, from **Miss D. Tharp (N), Dean's Office, Institute of Cancer Research, Haddow Laboratories, Costwold Road, Sutton, Surrey, SM2 5NG; Tel: 01-643-8901, ext. 4643.** (8389)F

UNIVERSITY OF GLASGOW
DEPARTMENT OF BIOCHEMISTRY
POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited as soon as possible for approximately 16 postgraduate studentships of various types tenable from 1st October, 1989 to work in the following research groups:—

Molecular Pharmacology
Biochemical Immunology and Parasitology
*Enzymology and Protein Engineering
Control of Gene Expression
Biochemical Endocrinology
*Plant Biochemistry and Molecular Biology
Structure and Function of Membranes
*Microbial Biotechnology
Cardiac and Vascular Biochemistry
Neurobiochemistry

Some of the projects that are available will involve collaboration with clinical departments and others will have strong industrial connections. The Department has excellent facilities for a very wide range of types of research.

Further information may be obtained from **Dr. J. G. Lindsay, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.** Telephone: 041-339 8855, Ext. 4720.

*Seven SERC 'earmarked' studentships have been awarded to individual staff members in the designated areas. (8404)F

THE UNIVERSITY OF SHEFFIELD

POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for postgraduate studentships tenable from 1st October 1989, or sooner.

The Department is multi-disciplinary and has excellent facilities for laboratory and clinical work. Research interests include studies on the control of secretion of pituitary hormones: the action of growth factors, cytokines and neurotransmitters: the control of calcium and phosphate metabolism in relation to bone disease; connective tissue biology and pathogenic mechanism in arthritis; and basic and clinical aspects of cancer research.

Topics available include the following:—

1. The development of biochemical methods of monitoring clinical disorders of bone and joint metabolism.
2. The origin, regulation and function of human bone cells *in vitro*, and the mode of action of steroid hormones and drugs on osteoblasts and osteoclasts.
3. Interactions between intracellular signalling systems (phosphoinositides, calcium and cyclic nucleotides) and the investigation of novel lipid mediators.
4. Studies on the role of neuroendocrine and paracrine agents (especially kinins and angiotensin) in the control of pituitary hormone secretion.
5. Studies on the genetic control and mechanisms of action of cytokines (e.g. interleukin 1, tumour necrosis factors, interferons and transforming growth factors) in human disease processes.

Applicants should possess a good honours degree in a relevant science subject. Candidates with previous work experience are particularly welcome to apply.

Applications should include a curriculum vitae and the names of two academic referees. Please apply to **Professor R. G. G. Russell, or Professor B. L. Brown, Department of Human Metabolism & Clinical Biochemistry, Beech Hill Road, Sheffield, S10 2RX (tel: 0742-766222, ext. 3037)** as soon as possible. (8405)F

KING'S COLLEGE LONDON
University of London
DIVISION OF BIOMEDICAL SERVICES
Anatomy and Human Biology

The Biological Sciences Committee of SERC has awarded an earmarked research studentship in support of a study into the regulation of cell adhesion and spreading. The project will investigate the role of oligosaccharide residues as modulators of the adhesion, and subsequent spreading, of cultured human cells onto purified glycoproteins of the extracellular matrix. The work will involve a wide range of techniques including protein purification and isolation of glycosylated and deglycosylated fragments, the use of monoclonal antibodies and synthetic peptide sequences which interfere with the adhesion mechanism, cell culture and cell adhesion assays, and computer-aided image capture and analysis techniques.

Final year students who expect to obtain a first class or upper second class honours degree in cell biology, biochemistry or a related discipline this summer are invited to contact: **Dr Gareth Jones, Anatomy and Human Biology, King's College London, Strand, London WC2R 2LS.** (8396)F

Cancer Research Campaign Laboratories
Immunology/Drug-Targeting Research Group
University of Nottingham NG7 2RD

POSTGRADUATE STUDENTSHIPS

Ph.D studentships are available commencing October, 1989 to participate in the departments research programme into immunological responses in cancer, including the use of anti-idiotypic antibodies (contact Dr. R.A. Robins) and tumour antigen biochemistry and structural studies relating to research on the development of monoclonal antibodies for drug targeting (contact Dr. M.R. Price).

Candidates must hold or expect to gain at least an upper second class honours degree. Further details may be obtained from the appropriate supervisor. Applications to be sent to the Secretary including two academic referees by April, 1989. (8391)F

AUSTRALIA

Australian Research Council

QUEEN ELIZABETH II FELLOWSHIPS

The Australian Government has established the Queen Elizabeth II Fellowships to encourage research by young scientists of exceptional promise and proven capacity for original work. Under this Scheme, 15 awards will be made annually. Fellowships may be taken up in industry, universities, colleges of advanced education or government research organisations in Australia and are normally for three years. Tenure of the Fellowships expected to commence within nine months of the announcement of the award.

Qualifications

Fellowships will be awarded to persons holding a PhD or equivalent qualifications, or equivalent research or professional experience. The decisions regarding equivalent qualifications will be made on a case by case basis. Recommendations for the award of Fellowships will be made to the Minister for Employment, Education and Training by the Australian Research Council. Fellowships will in general be restricted to applicants who are not more than 30 years of age when applications close, but consideration will be given to older people with less than five years post doctoral experience who have had an interrupted or late start to their academic career.

Eligibility

Fellowships will be awarded on merit to suitably qualified persons.

Salary

$31,143 (Australian) per annum increased to $33 738 per annum at age 28 years.

Travel Expenses

Necessary travel expenses are payable.

Research Support Grants

An additional supporting grant of up to $3 000 per annum to help cover research costs is available.

Applications

Persons interested in applying for the above Fellowships should obtain application forms and a statement of the Conditions of award from:

Director
Research Fellowships
Research Policy and Grants Branch
Department of Employment Education and Training
GPO Box 9880
CANBERRA ACT 2601 Australia

The next round of fellowships will be announced in October 1989. Applications received after the closing date of 14 April 1989 will not be considered.

(WF8876)E

Glaxo's Growth Is Your Opportunity

Glaxo Inc. is not only one of the Top 5 ethical pharmaceutical firms in America, we're also the fastest growing research-oriented company in the country. As part of our continuing growth, Glaxo is expanding its research facilities in Research Triangle Park, North Carolina to one million square feet, under the direction of Dr. Pedro Cuatrecasas. This has created opportunities for

POSTDOCTORAL FELLOWSHIPS

These positions, for a period of one to three years, will be available for highly qualified individuals with enthusiasm for discovery through basic research in the biological sciences. Publication of basic research findings is encouraged, as is participation in scientific meetings. Opportunities are available in the following areas:

- **modulation of ion channels using patch clamp technology**
- **regulation of fatty acid metabolism in inflammation**
- **role of free radicals in disease**
- **modulation of cell — cell interactions**
- **endothelial cell biology**
- **tumor progression and metastasis**
- **regulation of metabolism in diabetes and obesity**
- **in vivo cardiovascular pharmacology**

At Glaxo Inc. growth opportunities and state-of-the-art facilities are complemented by a location (Raleigh-Durham-Chapel Hill) that has excellent universities in close proximity. Further, we reward talent and creativity with an excellent salary and benefits package. Candidates interested in these positions, please send curriculum vitae, along with the names of three references, to:

Human Resources Dept.
Job #PF-S

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NEUROSCIENCE POSTDOCTORAL FELLOWSHIPS

Positions available to study GTP-binding proteins (second messengers, cytoskeletal proteins); excitatory amino acids (second messenger systems, proteolysis, toxicity, seizures); protein phosphorylation (esp. PKC, kinase activities, endogenous proteins, in vivo); cytoskeletal protein proteolysis and phosphorylation; seizurogenic, anticonvulsant, neurotoxic mechanisms; in rat CNS and neuronal cell lines.

Send description of experience, qualifications, interests and references to: **Dr. Richard Jope, Neuroscience Fellowship Program, Dept. Pharmacology, University of Alabama, Birmingham, AL 35294, USA.** (NW3314)E

nature

Please mention when replying to these advertisements

PHARMACEUTICAL SCIENCES INSTITUTE

DEPARTMENT OF PHARMACEUTICAL SCIENCES

Three Research Fellowships are available to support work in the Drug Development Group and the Cancer Research Campaign (CRC) Experimental Chemotherapy Group.

1. Postdoctoral Fellowship in Synthetic Chemistry (MRC AIDS Directed Programme)
Ref: 892/6

This three-year post will involve the design and synthesis of novel anti-viral agents specifically modified to facilitate their transport to the brain. The person appointed will be an organic or pharmaceutical chemist with an appreciation of the nature of the biological challenge of AIDS. The successful candidate will join a lively team employing modern techniques in the rational design of novel chemotherapeutic agents.

2. Postdoctoral Fellowship in Synthetic Chemistry (CRC Funded)
Ref: 894/6

The CRC Group seeks to appoint a chemist, preferably with experience in either stereo-controlled synthesis or modified peptide synthesis. The Fellow will join a large team of biologists, chemists and pharmacists with resources to develop novel agents from the chemistry bench through to clinical trial.

3. Postdoctoral Fellowship in Cell Biology (CRC Funded)
Ref: 893/6

The successful applicant will work on the growth and differentiation of murine colonic carcinomas in vitro. Cell lines have been established with variable degrees of differentation; the project seeks to investigate the effect of extracellular matrix components on the growth and differentation of these lines and to establish their chemosensitivity.

Commencing salaries will be within the range £9,865 to £15,720 per annum.

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff), quoting appropriate reference number, Aston University, Aston Triangle, Birmingham B4 7ET. Tel: 021 359 0870 (24 hour answer-phone).

Closing date for the receipt of applications is 10th March, 1989.

(8444)E

ASTON UNIVERSITY

THE ROYAL SOCIETY OF EDINBURGH

PERSONAL RESEARCH FELLOWSHIPS

The Royal Society of Edinburgh invites applications for a limited number of Personal Research Fellowships, tenable from 1 October 1989, to be held in Scottish Universities, Central Institutions, Colleges, Research Institutes and Industrial Laboratories.

Fellowships, which may be in any discipline, are held for up to three years and salaries will be on University Research grades 1A and 2 (in the range £12,150-£16,975). Applicants should normally be aged under 32, must possess a doctorate or equivalent higher qualification and must be able to demonstrate an outstanding ability for independent research.

Further details and application forms are available from the **Executive Secretary, The Royal Society of Edinburgh, 22-24 George Street, Edinburgh EH2 2PQ**, and must be returned by 31st March 1989. (8407)E

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Two Postdoctoral Fellowships

are available in molecular neurobiology, to join a multidisciplinary approach to the problem of neural regeneration in mammalian CNS (laboratories of Dr Frank Grosveld, Dr Roger Morris, Dr Geoff Raisman and Dr John Seely). A strong background in protein chemistry is preferred for one position, and in molecular biology/neurobiologoy for the second.

Positions are funded for 3 years by the International Spinal Research Trust, salary is according to MRC scales range £12,520–£15,950 inclusive of London Weighting per annum depending on experience.

Applications, including a curriculum vitae, and names of two professional referees, should be sent to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London NW7 1AA**, quoting job reference: ISRT5/PNB. Closing date for this post will be 10th March 1989.

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MRC Medical Research Council

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The Royal Society of Edinburgh

CASS Travelling Fellowships

The Royal Society of Edinburgh is pleased to be able to offer two new Travelling Fellowships in 1989 and invites commercial or industrial organisations in Scotland to nominate an employee whom they consider suitable for these prestigious Fellowships.

The Fellowships are intended to support travel abroad, for a period of normally up to three months, to study overseas research or commercial practices which would be of benefit to Scotland. Each Fellowship will be worth up to £5000, in addition to any contribution from the Fellow's employer. A requirement of these Fellowships is to produce a report, which will be published by the Society.

Nomination forms and further details can be obtained from the **Executive Secretary, Royal Society of Edinburgh, 22-24 George Street, Edinburgh EH2 2PQ** and must be returned by 28 April 1989. (8406)E

AWARDS

THE ROYAL SOCIETY OF EDINBURGH

AUBER BEQUEST FUND AWARDS

The Royal Society of Edinburgh invites applications for the Auber Bequest Fund Awards.

The Council of the Society is pleased to be able to offer to bona fide scholars and scientists assistance for the furtherance of academic research in any of its branches. To comply with the terms of the Auber Bequest applicants must:

1. be over sixty years of age
2. be naturalised British citizens or desire to acquire British nationality
3. reside in Scotland or England
4. be bona fide scholars engaged in academic research (but not industrial research)

The awards will normally be for a period of not more than twelve months and will not normally exceed £2,000. At the end of the period of the award, a report on the research carried out will be required and should be submitted to the Executive Secretary.

Application forms and further details are available from **The Executive Secretary, The Royal Society of Edinburgh, 22-24 George Street, Edinburgh EH2 2PQ.** Completed applications should be submitted to the Executive Secretary not later than 15 May 1989. (8408)N

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RSPB/Esso BIRDS AND COUNTRYSIDE AWARDS

More to the point, has someone you know done something for wild bird and countryside conservation? If so, the Royal Society for the Protection of Birds and Esso want to hear about it.

We are looking for individuals or organisations who have made an outstanding contribution to wild bird and countryside conservation in the following award categories:

INDIVIDUAL

For the person judged to have made the greatest contribution within the UK.

INDUSTRY

For the commercial body judged to have made the greatest collective contribution within the UK.

MEDIA

For the individual, publication or media organisation judged to have made the greatest contribution to the reporting of wild bird and countryside conservation issues.

EUROPEAN

For the individual or organisation judged to have made the greatest contribution in Europe, outside the UK.

GROUP

For the non-commercial, UK based body or authority judged to have made the greatest collective contribution within the UK.

ESSO AWARD

Top prize: £1,000

Two runners-up prizes: £500

This special award to be given to a young people's project (age range 9-16 years) judged by Esso to have made the greatest contribution to wild bird and countryside conservation, to give further support to their work.

Presentations will be made at a luncheon in London. Celebrities from the worlds of politics, industry, show business and conservation will be there to demonstrate their concern for our countryside. Will you?

For your RSPB/Esso Birds and Countryside Awards pack, telephone Margaret Gaylor on 0767 80551 or write to RSPB/Esso Birds and Countryside Awards, RSPB, The Lodge, Sandy, Beds SG19 2DL. Closing date for nominations is 31 March 1989.

RSPB

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ESSO

(8428)N

AGRICULTURAL & FOOD RESEARCH COUNCIL

FUNDING FOR PLANT MOLECULAR BIOLOGY

The AFRC has announced a major research initiative in plant molecular biology. Expenditure will be £3.5m in 1989/90, £4.5m in 1990/91 and £6m in later years. Applications are now invited for support.

Approximately half the funds will support a coordinated programme, principally in universities and polytechnics, on the molecular biology of plant development. The remainder will support research in universities, polytechnics and AFRC institutes on any aspect of plant molecular biology.

The coordinated programme will focus on *Arabidopsis thaliana*. This brassica relative has been chosen as a model system because the relative simplicity of its genome will facilitate the isolation of genes which have important functions in crop plants. The work will aim to understand the physical and genetical make up of this plant so that it will have greater genetic utility. Elements of research that will be encouraged include,

Development of protoplast and tissue culture methods to improve DNA transfer methods and to provide means for screening and selecting mutants.

Development of vectors for complementation of mutant phenotypes and homologous recombination.

Development of transposon tagging systems for identifying interesting genes.

Classical genetic studies in order to isolate and characterize new developmental mutants of *Arabidopsis*.

In support of the coordinated programme a library of overlapping clones covering the whole genome of *Arabidopsis* will be developed and maintained at the AFRC Institute of Plant Science Research.

In addition to plant molecular biologists, applications are strongly encouraged from geneticists, biochemists and other biologists who have not previously worked with plants but who would be interested in doing so.

Complementing the coordinated programme on *Arabidopsis*, research will be supported on plant molecular biology. This may be in any relevant area but the Council has identified the following topics as ones which it is particularly concerned to support.

Regulation of metabolic pathways

Regulation of development of any suitable species

Regulation of reproductive processes

Signal perception and transduction

Genome organisation and chromosome structure

Development of gene isolation, assessment and transfer techniques

Incorporation of commercially important genes into crop plants

Release of genetically manipulated organisms into the environment

Applications are now invited from UK Universities and Polytechnics to participate in the coordinated programme or in the separate research topics. Grants will normally be for three years in the first instance, but with the possibility of renewal. Support may be given for research and technical staff, equipment and consumables; the Council envisages significant expenditure on equipment. Where appropriate, applicants are also encouraged to make cases for funds to enable named overseas research scientists to work in this area in the UK for a period, and *vice versa*.

Further information on the programme may be obtained from Dr Gareth Price on 0793 514242, ext 255.

Applications, 5 copies, should be made (by 31 March 1989) on standard AFRC research grant forms addressed to, **AFRC Plant Molecular Biology Programme, University Support Branch, AFRC Central Office, Wiltshire Court, Farnsby Street, Swindon, SN1 5AT.** Telephone requests for forms should be made on 0793 514242 ext 206.

(8454)N

Call for Abstracts

THIRD ANNUAL NORTH AMERICAN CYSTIC FIBROSIS CONFERENCE

Tarpon Springs (Tampa), Florida
October 11–14, 1989

A multidisciplinary conference for researchers, physicians, and allied health professionals on the latest advances in CF research and care.

Plenary Sessions: The CF Gene—Where We Stand • Advances in Medical Treatment of CF • A Look to the Future

Symposia: Clinical Application of DNA Technology • The CF Gene—Molecular and Biochemical Approaches • CF 101 • Diagnosis and Management of Liver Disease • Heart-Lung Transplantation • Nutrition and CF • Management of Lung Disease in the First Two Years of Life • Controversies in CF Care • Successful Strategies for Living with CF

Basic Science Seminars: Pseudomonas Colonization and Persistence • Electrolyte Transport–Mechanisms and Regulation in CF

Conference proceedings will be published as a supplement to *Pediatric Pulmonology.*

For abstract forms and registration materials, contact the

Cystic Fibrosis Foundation

6931 Arlington Road
Bethesda, MD 20814 U.S.A.
(301) 951-4422

(NW3284)C

THE THIRD CAMBRIDGE MINI-SYMPOSIUM

(a series devoted to the exploration of the molecular and cellular aspects of drug actions).

JULY 7TH–8TH 1989
"RECEPTOR STRUCTURE AND FUNCTION"

Organised jointly by the Department of Pharmacology, University of Cambridge, the MRC Neurobiology Unit and the Parke-Davis Research Unit. To be held at Churchill College Cambridge.

In the 1989 Symposium we intend to explore the relationship between the structure and function of both membrane-bound and non membrane-bound proteins. The discussions are intended to range from X-ray and nmr data on receptor structure, enzyme and receptor-ligand interactions, through to functional responses involving, for example, linking to channels and regulatory proteins.

The audience and participants are intended to be multidisciplinary comprising of pharmacologists, biochemists and medicinal chemists.

Invited speakers include: T. Blundell (London), G. Wagner (Michigan), R. Schwyzer (Zurich), A. Fersht (Cambridge), J. Saunders (M.S.D.), P. Goodford (Oxford), P. Dean (Cambridge), A Walmsley (Leicester), C. Venter (Rockville), N. Unwin (Cambridge).

Attenadance is limited to invited participants selected from those applying as outlined below.

Poster Session. Posters are invited from participants. Please indicate your intention to present a poster and a suggested title with your application.

Registration details: The Registration Fee of £25 covers the meeting and accommodation at Churchill College on 7th July, lunches on 7th and 8th July, breakfast on 8th July and the Symposium Dinner on 8th July.

A limited number of students and post-doctoral workers within three years of qualification will be given free registration and accommodation. In exceptional circumstances, a travel bursary may be available. Applicants for these places and bursaries should enclose a letter of support from their Head of Department.

Please send applications by 21st April enclosing a cheque for £25 payable to Churchill College, Cambridge and a brief resume of your research interests to:

THE SYMPOSIUM SECRETARY
PARKE DAVIS RESEARCH UNIT
ADDENBROOKES HOSPITAL SITE
HILLS ROAD
CAMBRIDGE, CB2 2QB, U.K.

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UNIVERSITY OF LEICESTER

Department of Pathology and Anatomy and Continuing Education Unit

In-situ Hybridization : A Practical Course

11 – 15 September 1989

Course Leaders: Dr J. H. Pringle (Pathology) and Dr P. V. Senior (Anatomy).

The course covers the use of non-radioactive and radioactive in-situ hybridization for life science and clinical research. There will be an emphasis on practical "hands-on" experience in the following options:

1. The use of non-radioactive methods for the demonstration of cytomegalovirus using biotinylated and hapten labelled genomic DNA probes plus the use of biotinylated oligonucleotides to detect immunoglobulin mRNAs.
2. The use of radioactive probes to detect developmentally linked specific mRNAs for extracellular matrix proteins and insulin-like growth factors. This will include the use of ^{35}S labelled ^{SS} cRNA and ^{35}P labelled oligonucleotides.

Practical sessions will be designed to cover all aspects of the techniques and will extend over four working days. A comprehensive lecture programme, common to both practical options, will be provided in support of the laboratory sessions.

Fee £500 including full board and accommodation.

Closing date for applications: 12th June 1989.

Enquiries to: **Mrs Kate Penny**, tel (0533 522464), **Continuing Education Unit, University of Leicester, Leicester LE1 7RH, UK.** (8441)C

Royal Postgraduate Medical School
(University of London)

Immunology Update for Clinicians

A revision course for clinicians and scientists

10-19th May 1989

The course is divided into two parts: part A (days 1-3) provides an introduction to fundamental immunology, part B (days 4-8) is designed to bring participants up to date with current concepts and progress in this field and to relate these concepts to clinical problems. Students may attend either part, but the course is designed as a continuum. On receipt of the registration fee, all participants will be sent a booklet entitled "Basic Immunology" which is designed as a primer for the course.

Topics include:

Part A	*Part B*
Cells involved in the immune response	Receptors on lymphocytes
Cell surface molecules	Complement
Lymphocyte activation	Immune response to viruses
Monoclonal antibodies	Autoimmunity
The major histocompatibility complex	Transplantation
	Immunodeficiency

Speakers include:

Dr F Balkwill, Prof. J R Batchelor, Dr D Crawford, Dr J Lamb, Dr R I Lechler, Dr P Lydyard, Prof. A McMichael, Dr A J Pinching, Prof. J H Playfair, Dr A Rees, Dr M A Ritter, Prof. K Sikora, Dr J Weber, Dr A Webster

Course organisers Prof. J R Batchelor, Dr R I Lechler, Dr E R Kaminski, Dr M A Ritter

Course fee (inc. catering)
Part A: **£140** 3 days
Part B: **£235** 5 days
Parts A & B: **£365** 8 days

Further details from: Wolfson Conference Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN

Telephone: 01-740 3117

(7907)C

ontinued on page 28

President, C. Franzini

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DEADLINES

March 1, 1989:
Early registration
Receipt of Abstracts
Hotel Accomodation

(W5888)Q

IUPAC STOCKHOLM 1989

32nd IUPAC Congress

Stockholm 2-7 August 1989

Organized by the Swedish National Committee for Chemistry
The Royal Swedish Academy of Sciences

The Organizers cordially invite you to attend the 32nd Congress of IUPAC, the International Union of Pure and Applied Chemistry, which will be held from Wednesday 2 to Monday 7 August, 1989, in Stockholm, Sweden.

SCIENTIFIC PROGRAMME

The Scientific programme is mainly devoted to seven areas of chemistry, comprising the Sections described below.

The scientific programme will include plenary lectures, invited lectures, oral contributions and posters.

More than one hundred prominent lecturers from all over the world have been invited.

Contributions to all sections are welcomed, as well as general ones.

Section I Large-Scale Separation of Biological Macromolecules, Cells and Particles
Section II Atmospheric and Marine Chemistry
Section III Chemical Communications and Chemical Interaction between Organisms
Section IV Solid State Chemistry — Frontiers in the Chemistry of Inorganic Materials
Section V Structure and Dynamics of Macromolecules
Section VI Electron Transfer Reactions
Section VII Chemistry and Biochemistry of Bile Acids

Name .....
Institution/Company .....
Department .....
Address .....
City .....
Country .....

For further information mail to: 32nd IUPAC Congress, c/o Stockholm Convention Bureau, P.O. Box 6911, S-102 39 Stockholm, Sweden.

(W5878)Q

1ST INTERNATIONAL WORKSHOP ON THE MOLECULAR AND CELL BIOLOGY OF AUTOANTIBODIES AND AUTOIMMUNITY

July 27–July 29, 1989
at the Heidelberg Academy of Sciences
Heidelberg, Federal Republic of Germany

Organized by
E. K. F. Bautz, Heidelberg, **J. R. Kalden**, Erlangen, **M. Homma**, Tokyo, **E. M. Tan**, La Jolla

There will be five half-day sessions on

- **Clinical Autoimmunity**
- **Small Nuclear RNP's**
- **Synthetases, Polymerases and DNA-Binding Proteins**
- **Recombinant Autoantigens**
- **Cytoplasmic Antigens**

Each session will be introduced by a keynote speech followed by oral- and poster-presentations. The organizers invite submission of abstracts concerning recent work for oral or poster presentations. Attendance at the workshop is limited to 100 participants and is free of charge.

Applications for attendance and abstracts of one to two pages should be sent not later than April 30, 1989 to:

Prof. Dr. E. K. F. Bautz
Institute of Molecular Genetics
University of Heidelberg
Im Neuenheimer Feld 230
6900 Heidelberg/West Germany
Tel.: 6221-562677/Fax: 6221-562678

(W5872)V

NATO Advanced Research Workshop
REGULATION OF EXTRAVASCULAR FIBRINOLYSIS IN NERVOUS SYSTEM DEVELOPMENT AND DISEASE

July 2–8, 1989
Acquafredda d1 Maratea, Italy

Speakers include:

K. Dano (Copenhagen)
N. Beeds (Denver)
D. Monard (Basel)
H. Lijnen (Louven)
E. Levin (La Jolla)
D. Cunningham (Irvine)
J. Fenton (Albany)
R. Pittman (Philadelphia)
R. Tanzi (Boston)
J. Baker (San Francisco)
F. Blaai (Copenhagen)
R. Hill (Edinburgh)

The Workshop will consist of lectures, discussion and poster sessions. Attendance is limited and only by invitation. Several places have been reserved for participants other than speakers. Deadline for application and abstract (not exceeding 1 page) is April 13, 1989, please include FAX number, if any. Applicants will be notified by May 1, 1989. Cost for board and lodging is $500 (double occupancy). Limited NATO funds available for selected applicants from NATO countries. No registration fee.

Abstracts and letters of application with curriculum vitae to:

Dr. Barry W. Festoff (Workshop Director)
Neurobiology 151
VA Medical Center
4501 Linwood Boulevard
Kansas City, Missouri 64128 USA

Organizing Committee: Barry W. Festoff, Director (Kansas City, USA); Daniel Hantaï (Paris, France); Georgia Barlovatz-Meimon (Paris, France); Robin Carrell (Cambridge, U.K.); Gustave Moonen (Liege, Belgium).

(NW3319)V

COURSES & CONFERENCES

METHODS IN COMPUTATIONAL NEUROSCIENCE
August 6 – September 2, 1989

This intense, advanced lecture and laboratory course presents the basic techniques necessary to study single cells and neural networks using simulation software developed at the California Institute of Technology. Limited to 20 students. Co-Directors: James M. Bower and Christof Koch, California Institute of Technology. Applications deadline: May 15th, 1989

For further information and application forms, contact: **Admissions Coordinator, Marine Biological Laboratory, Woods Hole, MA 02543, Tel. (508) 548-3705, Ext. 216.**

(NW3311)C



EMBL

MOUSE MOLECULAR GENETICS

AUGUST 26 – 30, 1989
ORGANISED BY:

Erwin Wagner, IMP, Vienna
Peter Gruss, MPI, Göttingen
Richard Palmiter, HHMI, Seattle

This is the second in a series of annual meetings the first of which was held in 1988 at CSH, focusing on the molecular biology and molecular genetics of the mouse.

PROPOSED SESSIONS:

Embryonic Stem Cells
Cell Proliferation and Oncogenesis
Development of the Hemopoetic and Nervous Systems
Regulation of Gene Expression
Molecular Characterisation of Mutations
Molecular Genetics of Development
Molecular Approaches to Physiology
Models of Disease Processes

All prospective participants should submit abstracts within the context described above by **May 15 1989**
Abstracts will be selected for presentation as either talks or posters. There will be a small number of invited talks, as well as posters and short talks selected from abstracts. Graduate students and postdoctoral fellows are encouraged to apply

Registration, food and housing will be **DM 600.-**
Reduced graduate students rate **DM 400.-**
Requests for additional registration and/or abstract materials should be forwarded to Erwin Wagner, IMP, Dr. Bohr-Gasse 7, 1030 Vienna, Austria
Tel.: (222) 79 26 36-654, Fax: (222) 78 71 53

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11TH INTERNATIONAL CONGRESS OF THE INTERNATIONAL SOCIETY OF DEVELOPMENTAL BIOLOGISTS 20TH-25TH AUGUST 1989

JAARBEURS CONGRESS BUILDING UTRECHT THE NETHERLANDS

YOUR OPPORTUNITY TO REVIEW ADVANCES IN THE FIELDS OF HUMAN, ANIMAL AND PLANT DEVELOPMENT

Developmental biology is a rapidly advancing science which enjoys increasing attention from a wide range of associated disciplines. The programme for this major scientific event will place emphasis on assessments in a wide variety of disciplines, including molecular biology, cell biology, biochemistry, embryology, genetics, immunology, botany, oncology and biotechnology. Plenary lectures, parallel symposia, poster contributions and special social events will combine to make the congress an international success.

Chairpersons		Symposia topics include:
R.G. Edwards	Cambridge	In vitro fertilisation and embryo transplantation
W.J. Gehring	Basel	Genetic control of pattern formation
Th. Graf	Heidelberg	Haemopoietic stem cell differentiation
F. Gros	Paris	Muscle development
J.B. Gurdon	Cambridge	Embryonic induction
J.K. Heath	Oxford	Growth factors and their receptors
M. Johnson	Cambridge	Cytoplasmic organisation and development
G. Klein	Stockholm	Developmental tumors
N. LeDouarin	Nogent-sur-Marne	Developmental neurobiology
A. McLaren	London	Germ cells
F. Melchers	Basel	Developmental immunology
H. Mohr	Freiburg	Developmental botany
G.B. Pierce	Denver	Teratocarcinoma and early mammalian development
J. Rossant	Toronto	Cell lineage in early embryogenesis
H.C. Schaller	Heidelberg	Pattern formation in invertebrates and lower eukaryotes
J. Sedat	San Francisco	Nuclear organisation and gene expression
A. Surani	Cambridge	Genomic imprinting and early development
J.P. Thiery	Paris	Extra-cellular matrix and cell adhesion molecules
M.Van Montagu	Ghent	Molecular development of plants
I.M. Verma	San Diego	Signal transduction and transcriptional regulation
E. Wagner	Heidelberg	Gene transfer into stem cells and embryos

International Exhibition: the publishers of *Nature* will be organising an associated international exhibition.

Poster sessions open to all participants: Deadline for abstracts 15th April 1989.

GRANTS AVAILABLE

The organising committee has a limited amount of grant money available for young scientists, under the age of 35, wishing to attend the meeting. Applications including a curriculum vitae and an abstract to be sent to:

ISDB Congress, c/o Hubrecht Laboratory,
Uppsalalaan 8, 3584 CT Utrecht,
The Netherlands.
Tel: 31 30 510211, Fax: 31 30 516464

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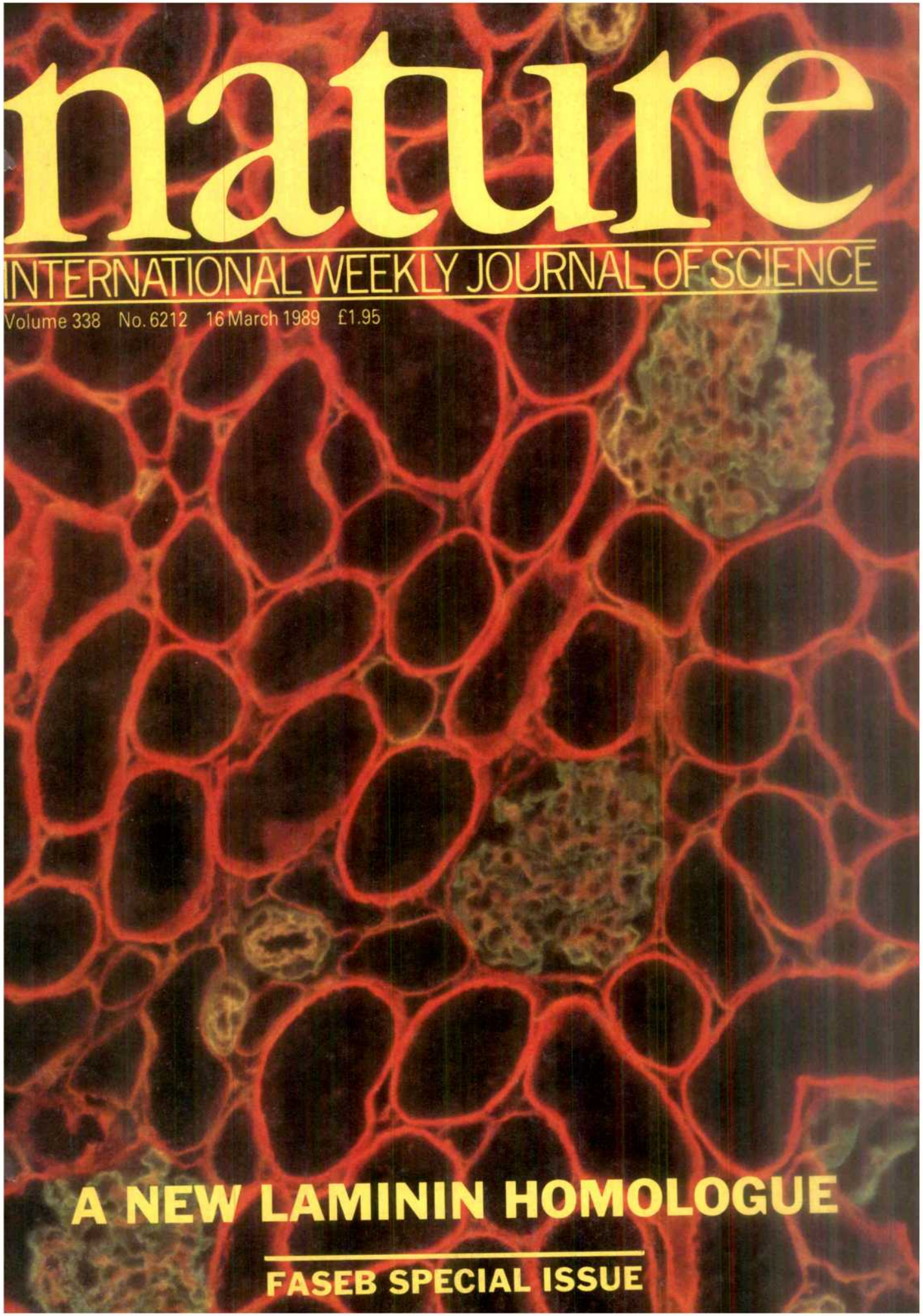
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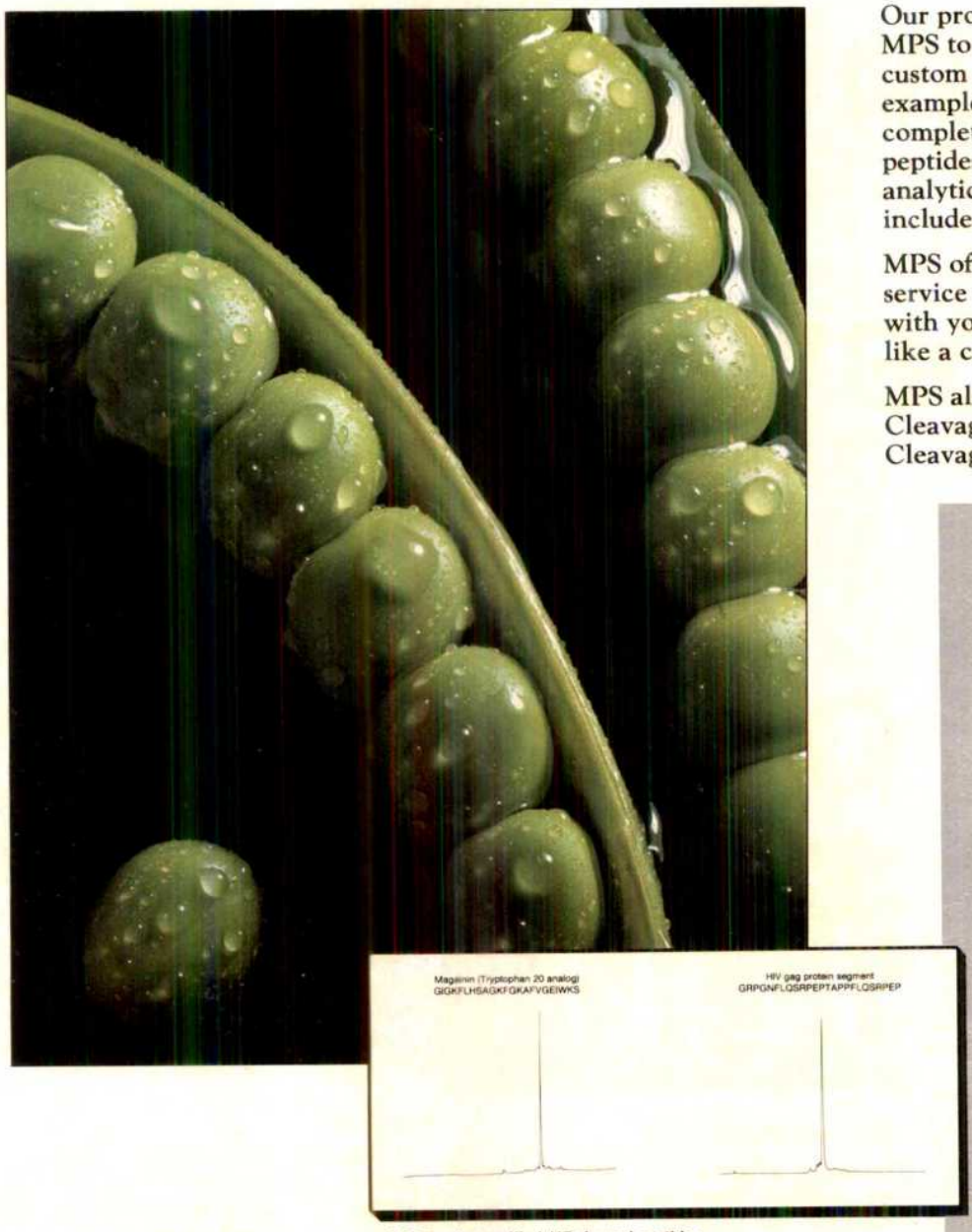
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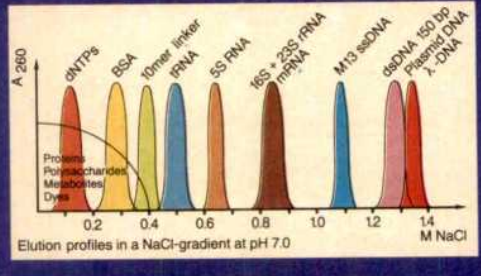
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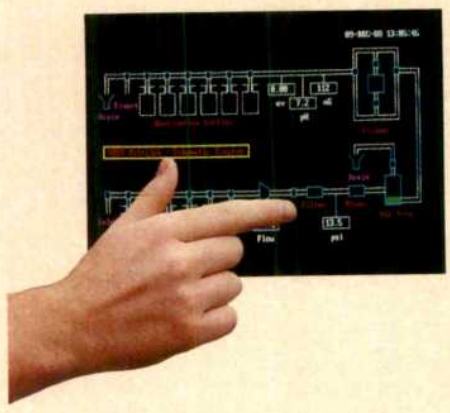
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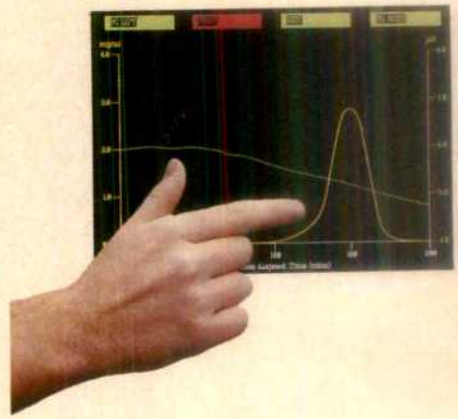
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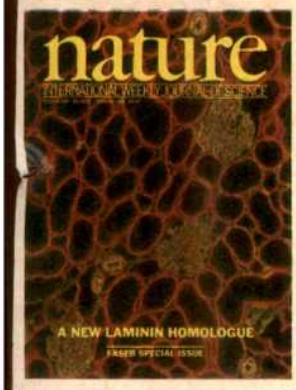
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nature

16 March 1989

Vol. 338 Issue no. 6212

S-laminin, like its homologue laminin, is found in basement membranes but with more limited distribution, including neuromuscular junctions (see page 229). The cover shows an immunostained section of kidney with s-laminin (green) in the glomeruli and laminin (red) in both tubules and glomeruli.

THIS WEEK

Radiation risks

Reassessment of the exposure of the British public to indoor radon gas shows radon to be an even greater hazard than previously thought; on average, people are exposed to over 1,000 times the radiation dose from radon than from radioactive effluent discharges. Commentary, page 197.

Uncertain origins

Gerald Joyce reviews the evidence that at an early stage in evolution life was based on RNA. Although the evidence is compelling, understanding how an 'RNA world' could have come about poses many problems. Page 217.

Celestial mechanics

A numerical integration extending backwards over 200 million years suggests that the motion of the Solar System is chaotic and that the predictability of planetary orbits, including the Earth's, would be lost within a few tens of millions of years, pages 237 and 207. And a clay tablet, found in 1948 among the ruins of ancient Ugarit, in what is now Syria, may be a record of an eclipse that occurred on 5 March 1223 BC, 152 years earlier than previously thought, with important implications. Pages 238 and 204.

Dunnock behaviour

DNA fingerprinting has been applied to the hedge sparrow *Prunella modularis*, a small passerine bird with a variable mating system, to relate parental care and mating system to paternity, pages 249 and 203.

Anionic doping

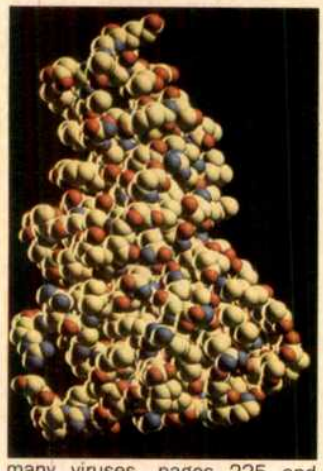
The first high-temperature superconductor formed by anionic rather than cationic substitution is reported on page 240.

New pulsar not alone

The discovery a few weeks ago of a submillisecond pulsar in supernova 1987A has turned theory on its head. The apparent 8-hour periodicity, on top of the 2,000-Hz pulsation rate, may indicate a Jupiter-mass companion in a one-million-kilometre orbit, or may be due to some instability in the neutron star itself. Page 234.

'Jelly-roll' protein

The crystal structure of the cytokine tumour necrosis factor (TNF) shows it to be a trimer of subunits with the 'jelly roll' fold characteristic of the coat proteins of



many viruses, pages 225 and 205.

FASEB Special Issue

Papers of particular interest to delegates at the New Orleans meeting of the Federation of American Societies for Experimental Biology include: AIDS in San Francisco (p.251); transgenic rice (p.274); generation of antibodies with specific catalytic properties (p.269, p.206); oestrogen receptors (p.271) and genetic footprinting (p.277). On page 278, news of exhibits at the FASEB exposition.

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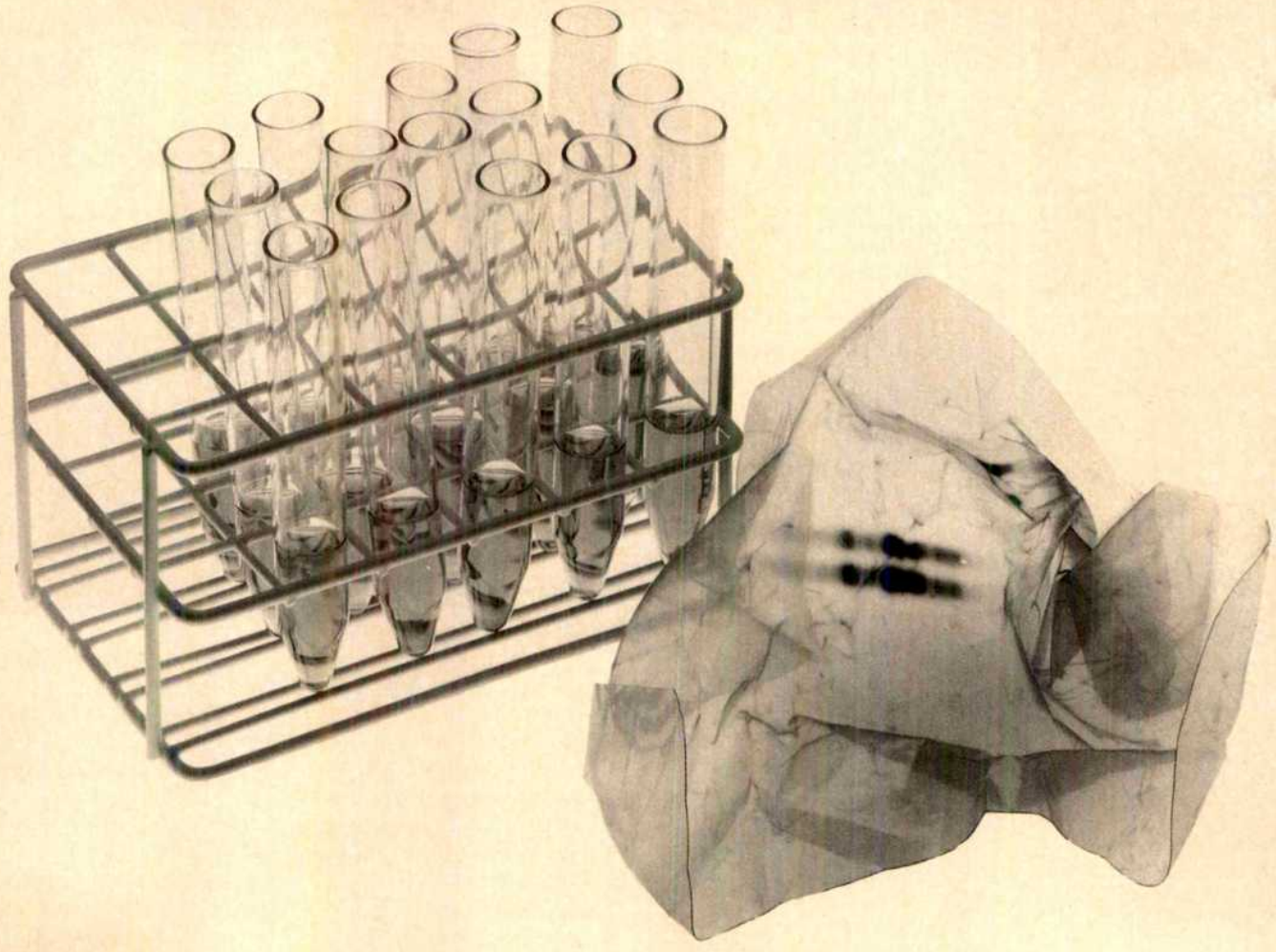
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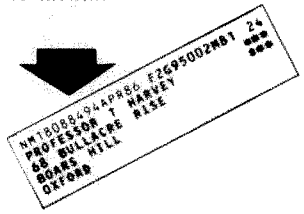
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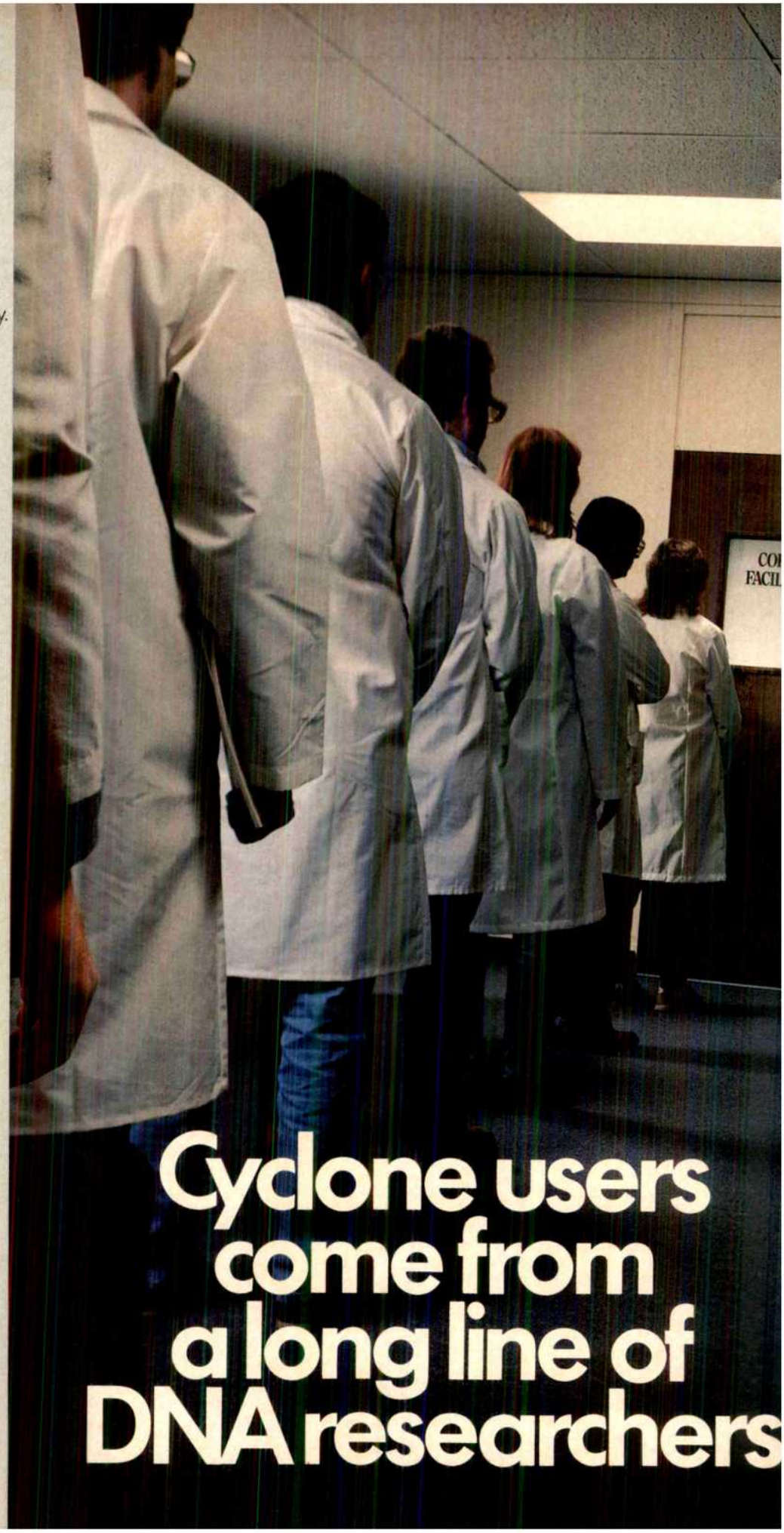
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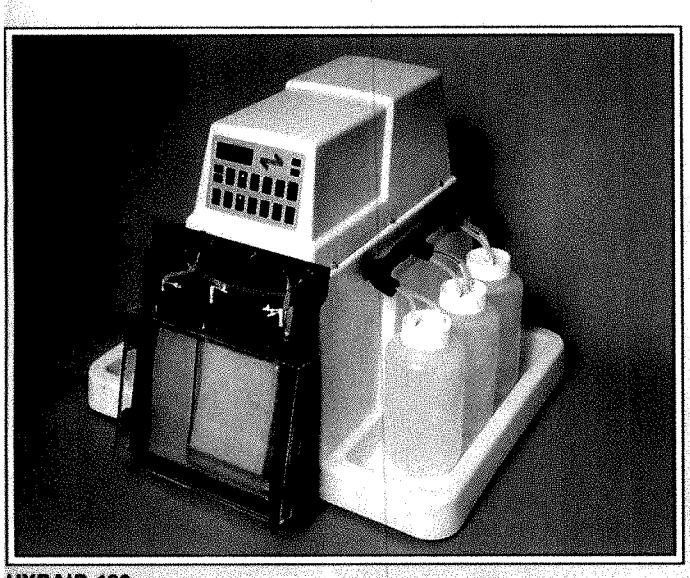
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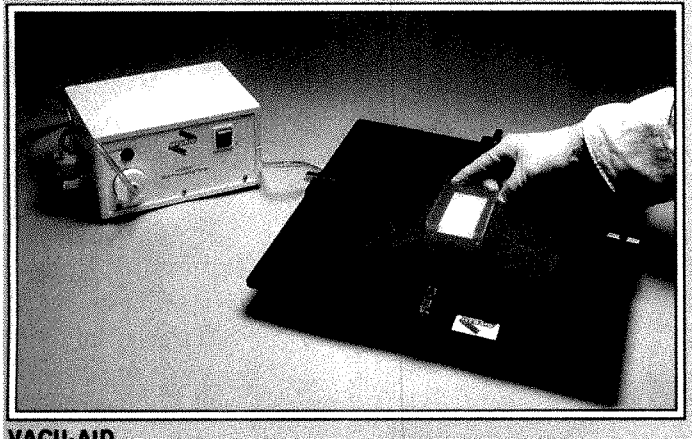
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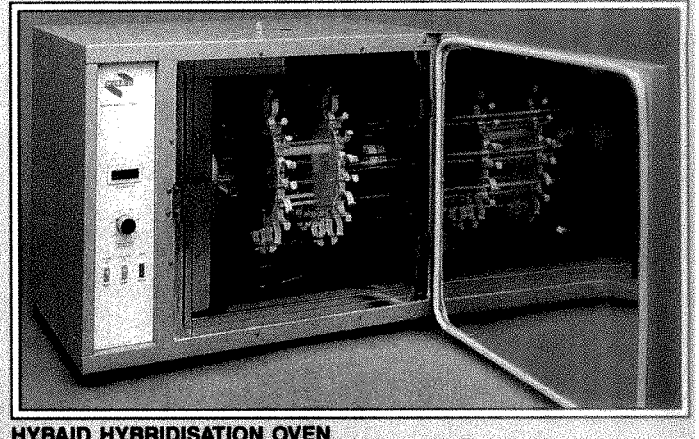
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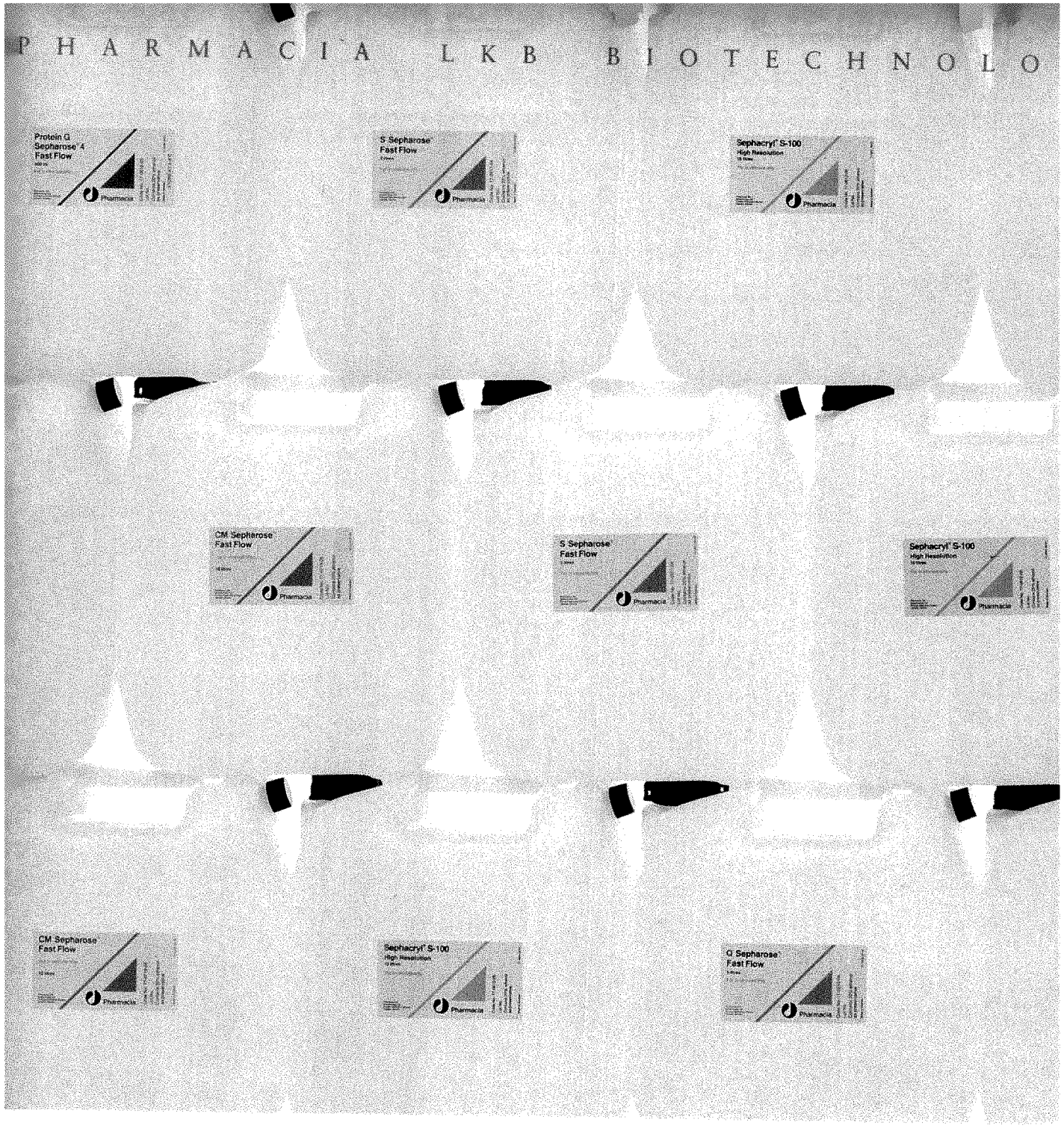


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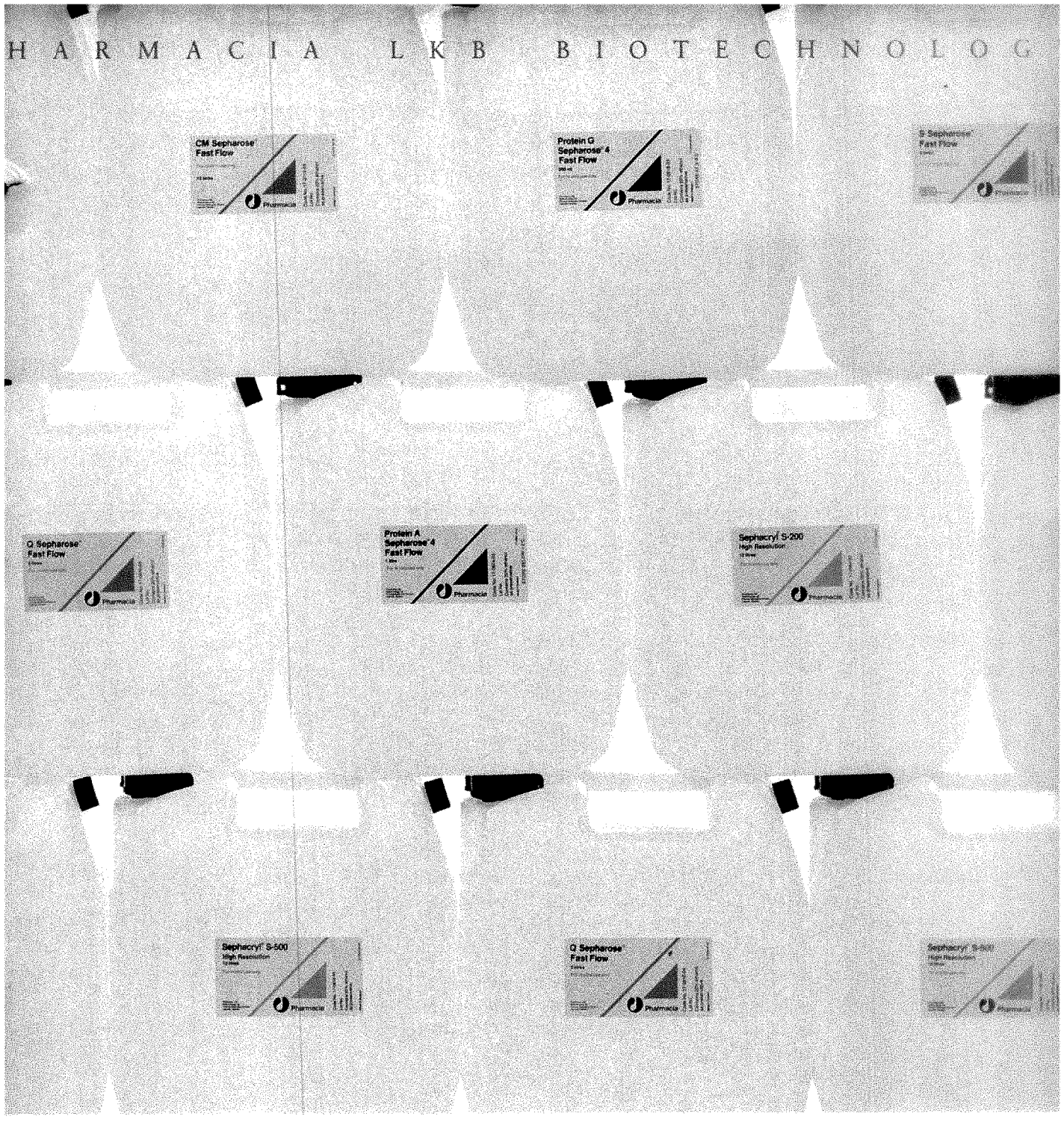
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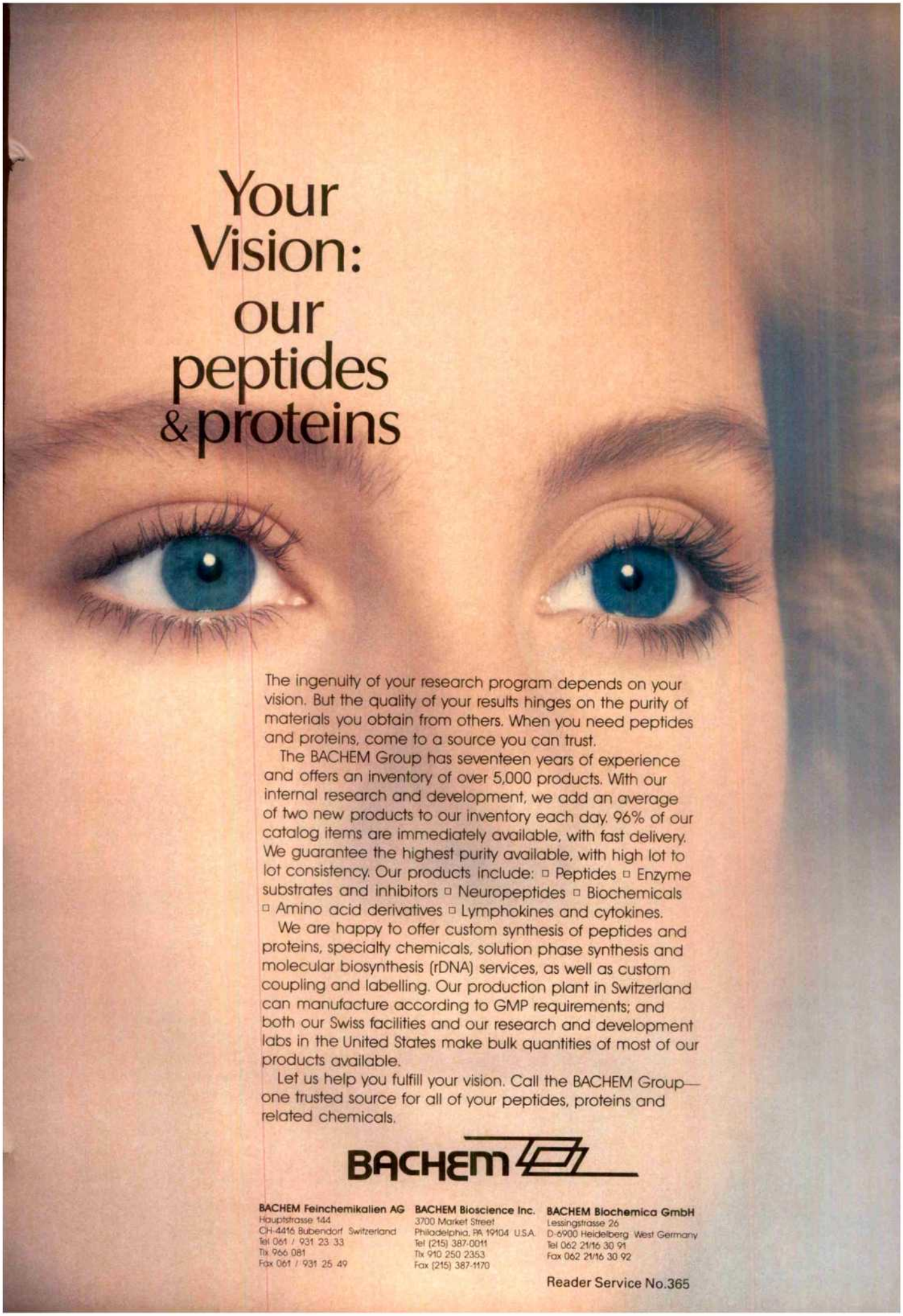
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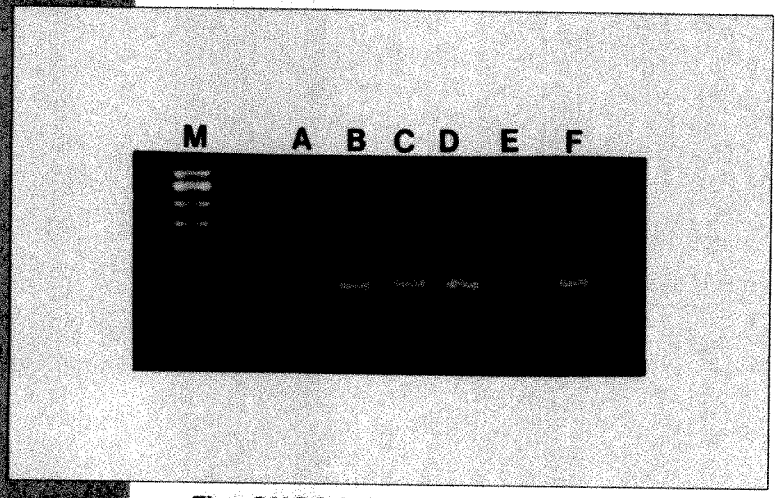
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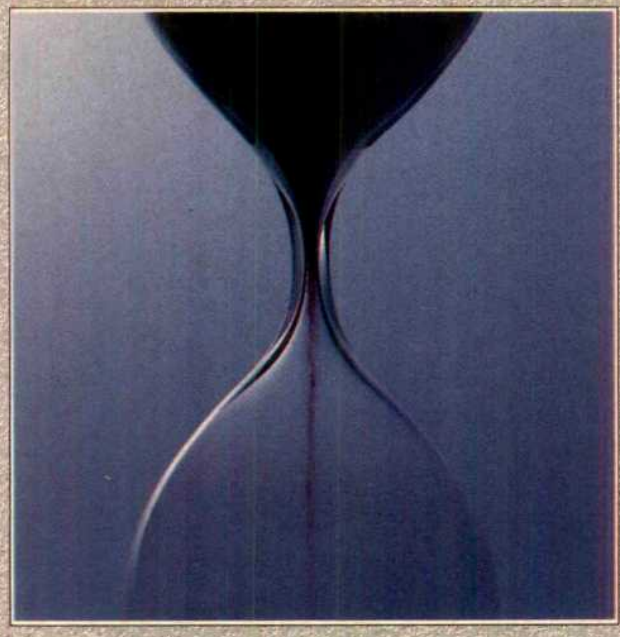
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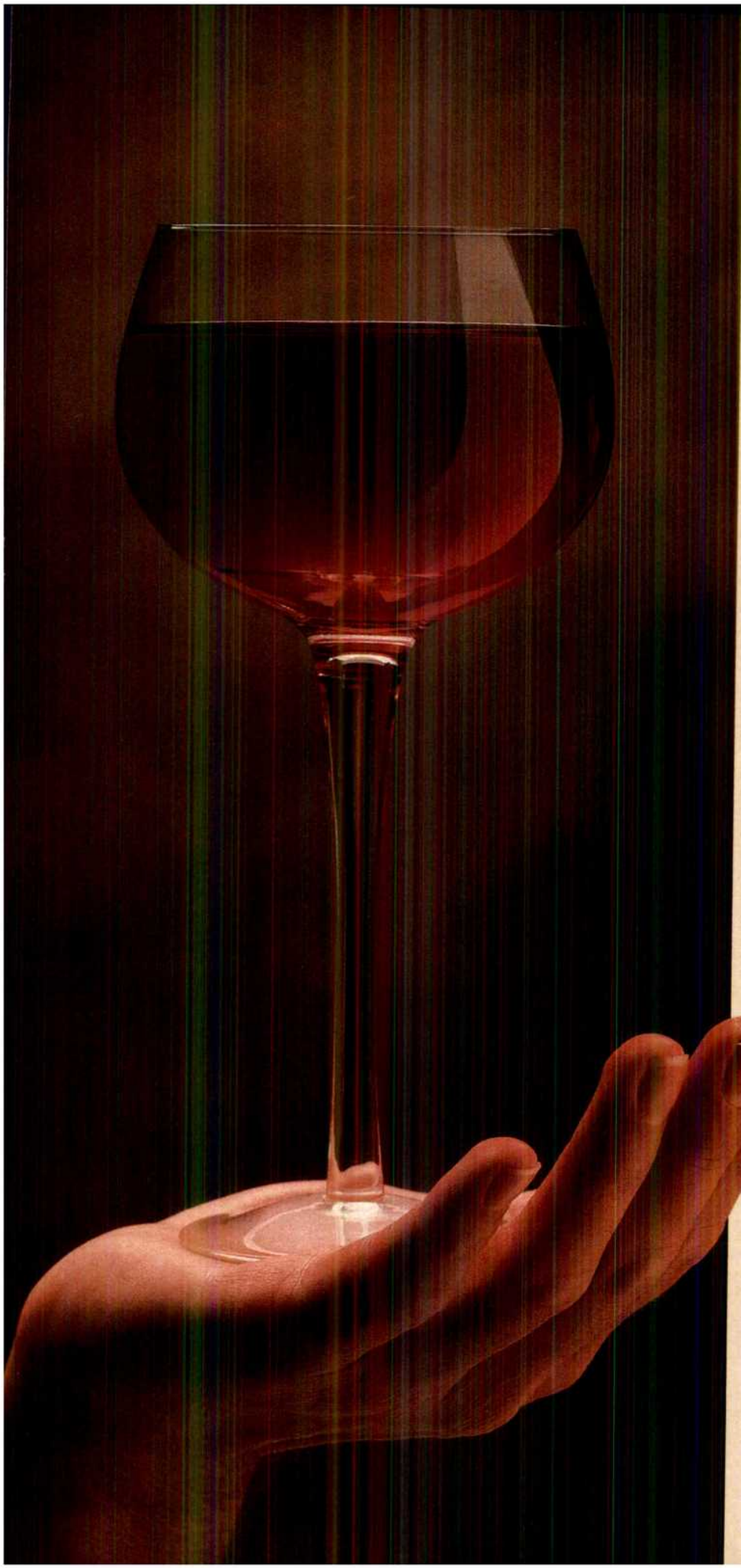
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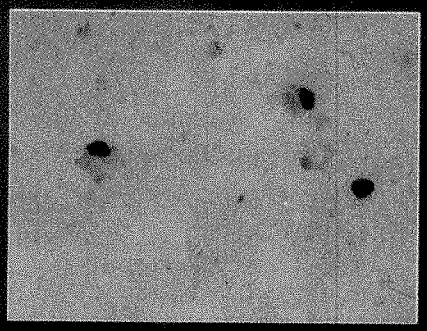
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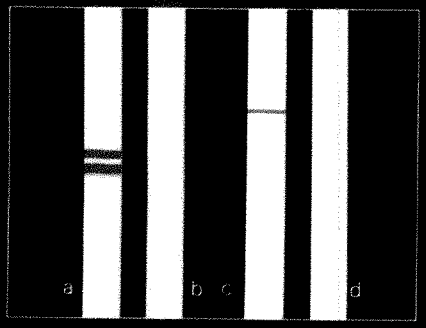
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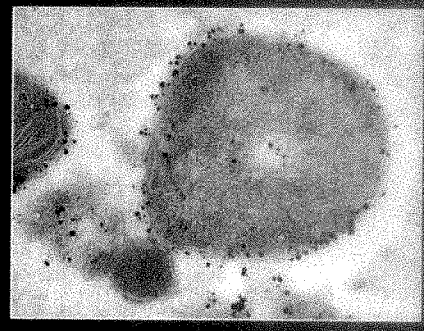
← *In situ* hybridization of cytomegalovirus (CMV)-infected adrenal gland using biotinylated entire CMV genome, rabbit anti-biotin, AuroProbe One and IntenSE.
(Volkers, van den Brink & Houthoff, Amsterdam)



Cryosectioned synaptosomes reacted with rabbit anti-B50, AuroProbe One and IntenSE.
(van Lookeren-Campagne & van Bergen en Henegouwen, Utrecht) →

← Blot of total extract of Hela cells and blot of partially purified nuclei of rat liver reacted respectively with rabbit anti-tubulin (a) and rabbit anti-actin (c) followed by AuroProbe One and IntenSE. Negative controls (b, d)

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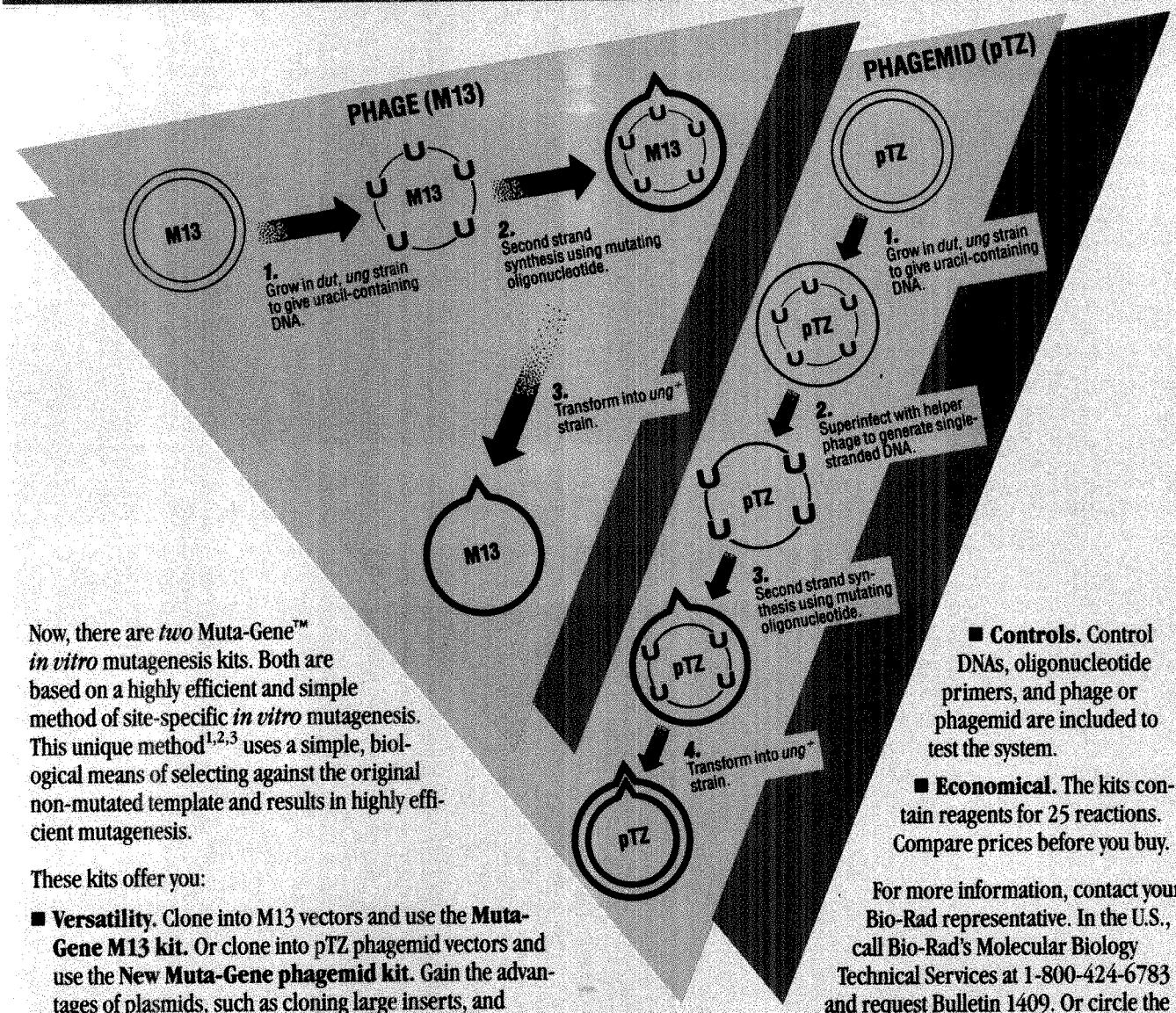
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[1] Kunkel, T.A. *Proc. Natl. Acad. Sci. USA* **82**, 488-492. 1985.
[2] Kunkel, T.A., Roberts, J.D., and Zakour, R.A. *Meth. Enzymol.* **154**, 367-382. 1987.
[3] Patent pending.
[4] Mead, D.A., Szczesna-Skorupa, E., and Kemper, B. *Prot. Eng.* **1**, 67-74. 1986.

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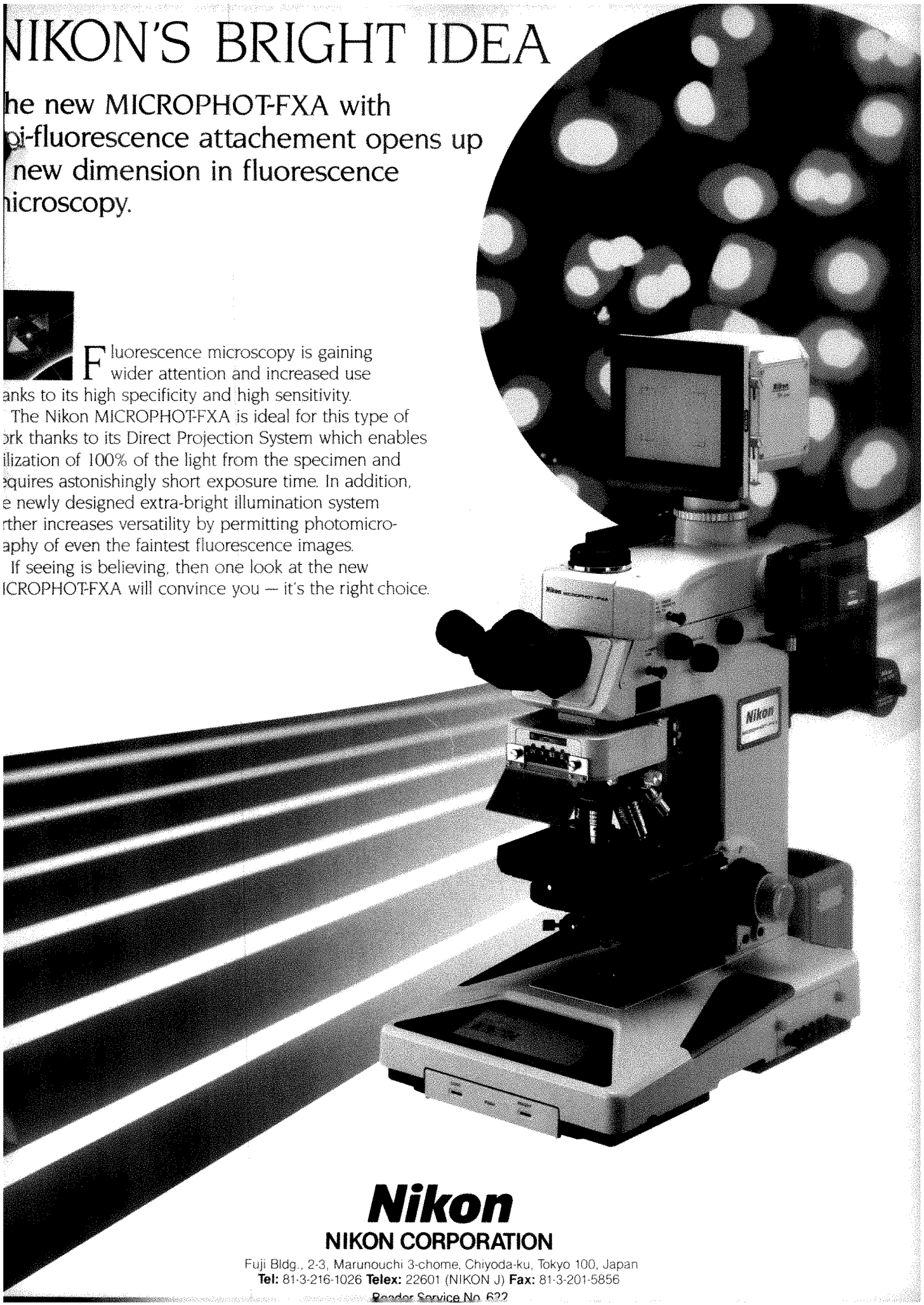
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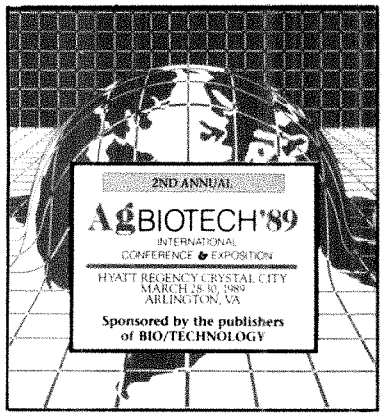
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PROGRA

Tuesday, March 28

BUSINESS FORUM

8:00 a.m. – 11:00 a.m.

Management Strategies
Market Sizes and Structures
Long Range Strategic Planning

Chair: David Wheat
Panelists: A. Barnes, G. Kidd, R. Laster

11:00 a.m. – 2:00 p.m.:

2:00 p.m. – 5:00 p.m.

Management Strategies
International Strategic Alliances
High Level Staffing

Chair: Kelly Kincannon
Panelists: C. Baker, J. Bouckaert, J. Marc

Wednesday, March 29

8:00 a.m. – 11:00 a.m.

Regulation: Case Studies; Testing for Registering Microbial Pesticides

Chair: Edgar R. Butts and Robert B. Nicholas
Panelists: D. Glass, C. Hutchinson, R. Kahn, J. Panetta, F. Serdy, J. Swigert, S. Woodhead

11:00 a.m. – 2:00 p.m.:

2:00 p.m. – 5:00 p.m.

Commercial Financing

Chair: G. Steven Burrill
Panelists: C. Earl, R. Moshe, L. Stern, D. Wagster

Thursday, March 30

8:00 a.m. – 11:00 a.m.

Patents: Animal; Plant

Chair: Kathleen Merrigan
Panelists: D. Beier, A. Douglas, J. Doyle, H. Lyman, L. McKenzie, K. O'Conner, R. Quisenberry

11:00 a.m. – 2:00 p.m.:

1:30 p.m. – 4:30 p.m.

Government Research Funding Policies

Chair: Gary B. Ellis
Panelists: R. Dull, V. Giddings, K. Hanna, W. Marshall, M. Phillips, J. Tavares

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AT A GLANCE

SCIENCE AND TECHNICAL FORUM

Tuesday, March 28

ant Molecular Biology	Animals
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.
: Production of Transgenic Crops Dicots – Cotton, Soybean, Oil Seed Rape, Walnut Pine air: Harvey Bialy nelists: K. Barton, A. Dandekar, illatti, M. Hinchee	Molecular Strategies for Production Animal Improvement: Transgenes, Genome Mapping **Chair:** Charles Arntzen
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS	
2:00 p.m. – 5:00 p.m.	2:00 p.m. – 5:00 p.m.
: Production of Transgenic Crops Monocots – Rice, Maize, Asparagus air: Indra Vasil nelists: R. Shillito, M. Von Montagu, Wu	Molecular Strategies for Production Animal Improvement: Transgenes, Genome Mapping (continued) **Chair:** Charles Arntzen

Wednesday, March 29

ant Molecular Biology	Animals
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.
technology, Plant Breeding and Crop provement air: Marc Von Montagu nelists: H. Donner, T. Helentjaris, rivalle, I. Vasil	Animal Healthcare Vaccines Diagnostics Immune Stimulation **Chair:** Fred Brown **Panelists:** G. Ada, J. Cantrell, J. McMillen
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS	
2:00 p.m. – 5:00 p.m.	2:00 p.m. – 5:00 p.m.
ntrolling Gene Expression air: Harvey Bialy nelists: L. Comai, R. Fuchs, C. Lamb	Animal Healthcare (continued) **Chair:** Fred Brown **Panelists:** G. Ada, J. Cantrell, J. McMillen

Thursday, March 30

Perspectives	Biopesticides
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.
rogen Fixation air: Ethan Signer nelists: D. Kahn, D.P. Verma, G. Walker	Biopesticides Bacillus thuringiensis Endophytes Baculovirus **Chair:** Jerry Caulder
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS	
1:30 p.m. – 4:30 p.m.	1:30 p.m. – 4:30 p.m.
: International Picture air: Mark Ratner nelists: J. Cohen, P. Oram, W. Roca, Sondahl, G. Toenniessen	Biopesticides (continued) **Chair:** Jerry Caulder

AgBIOTECH '89 Faculty Continued:

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Janis McMillen, SyntroVet
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Suzan H. Woodhead, Ricerca, Inc.
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Exhibit Hall Hours

Tuesday, March 28 —
11 a.m. to 5 p.m.

Wednesday, March 29 —
11 a.m. to 5 p.m.

Thursday, March 30 —
11 a.m. to 3 p.m.

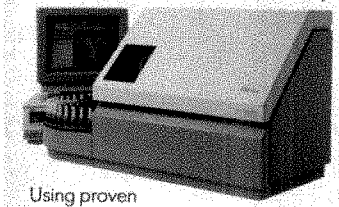
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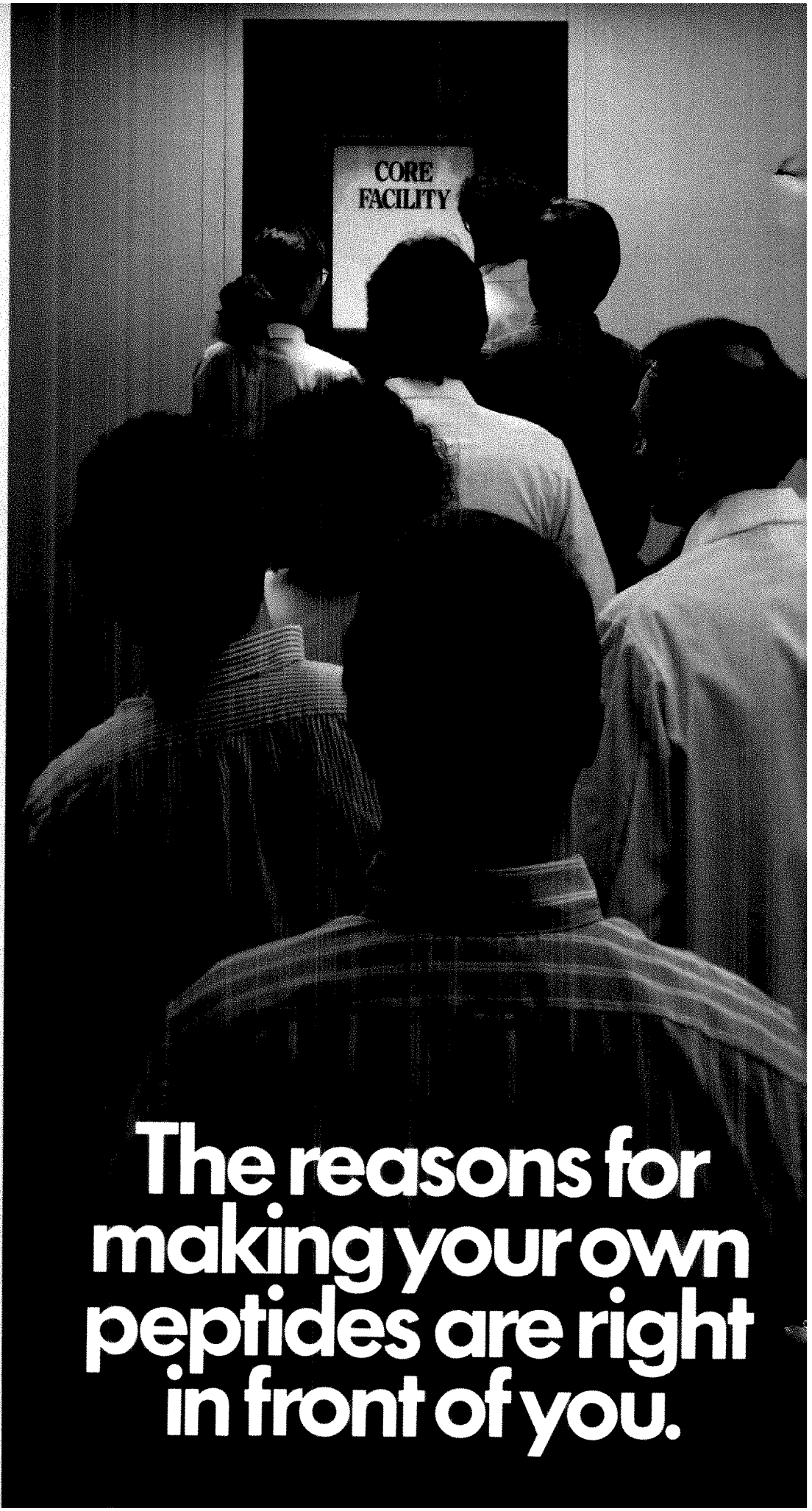
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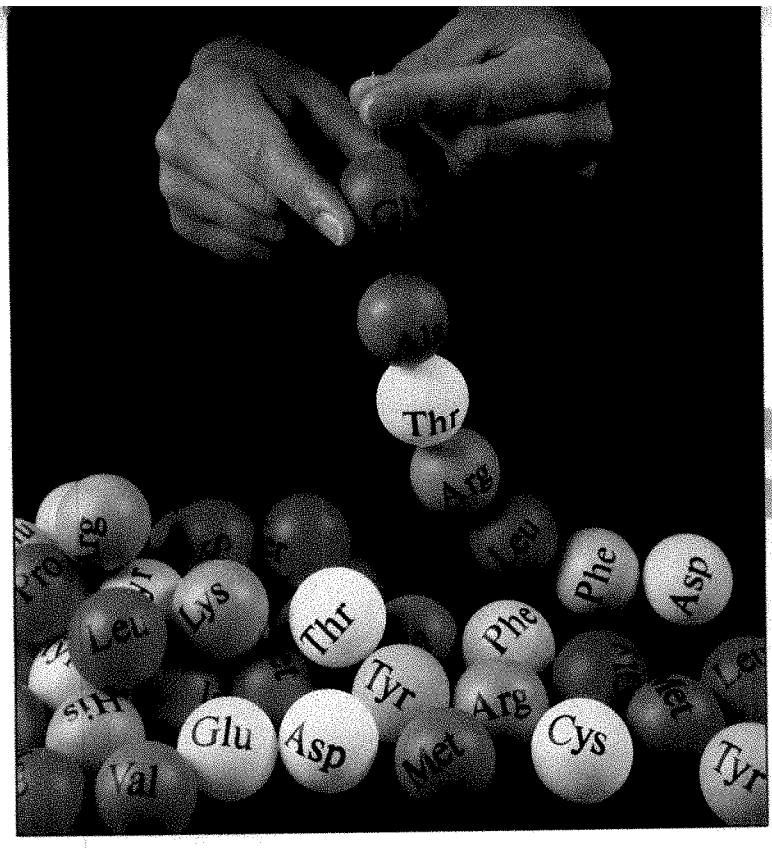
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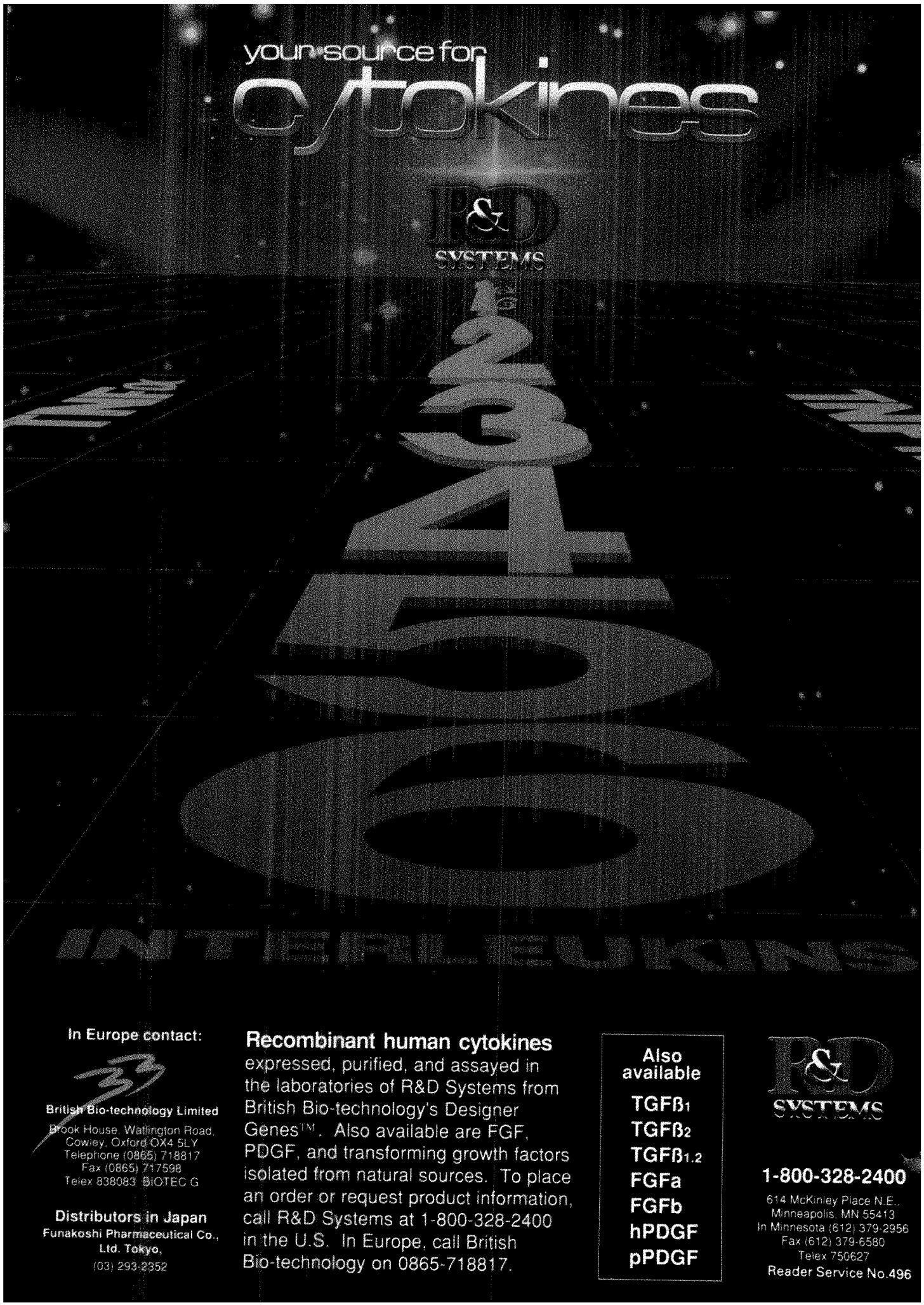
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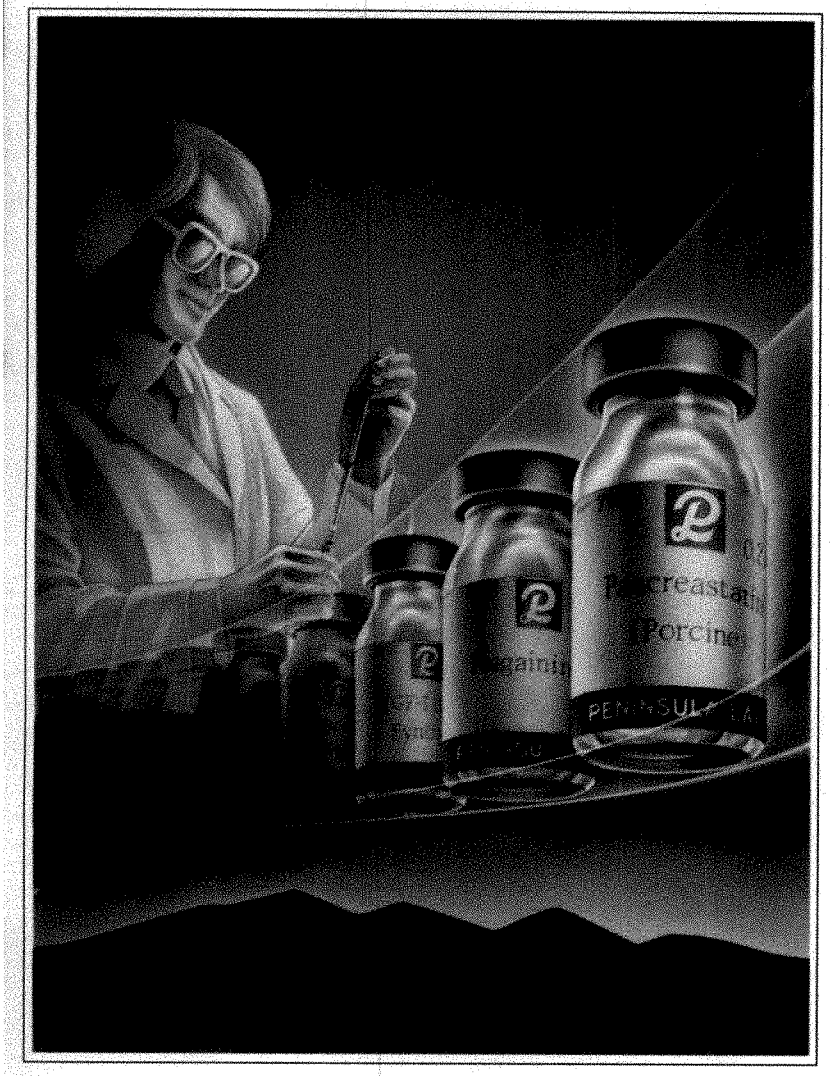
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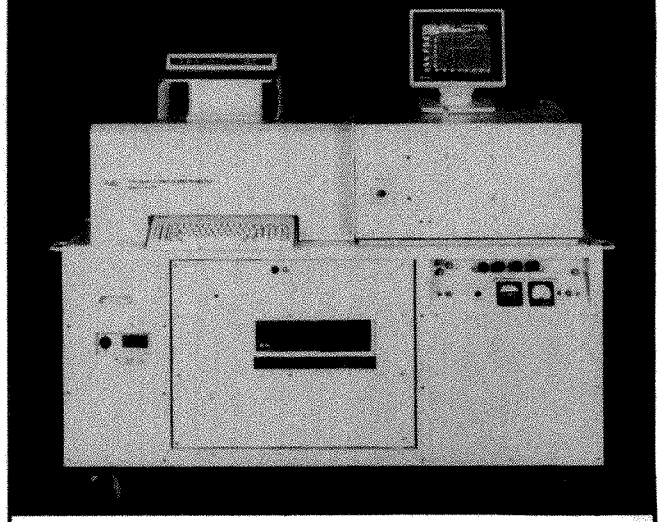
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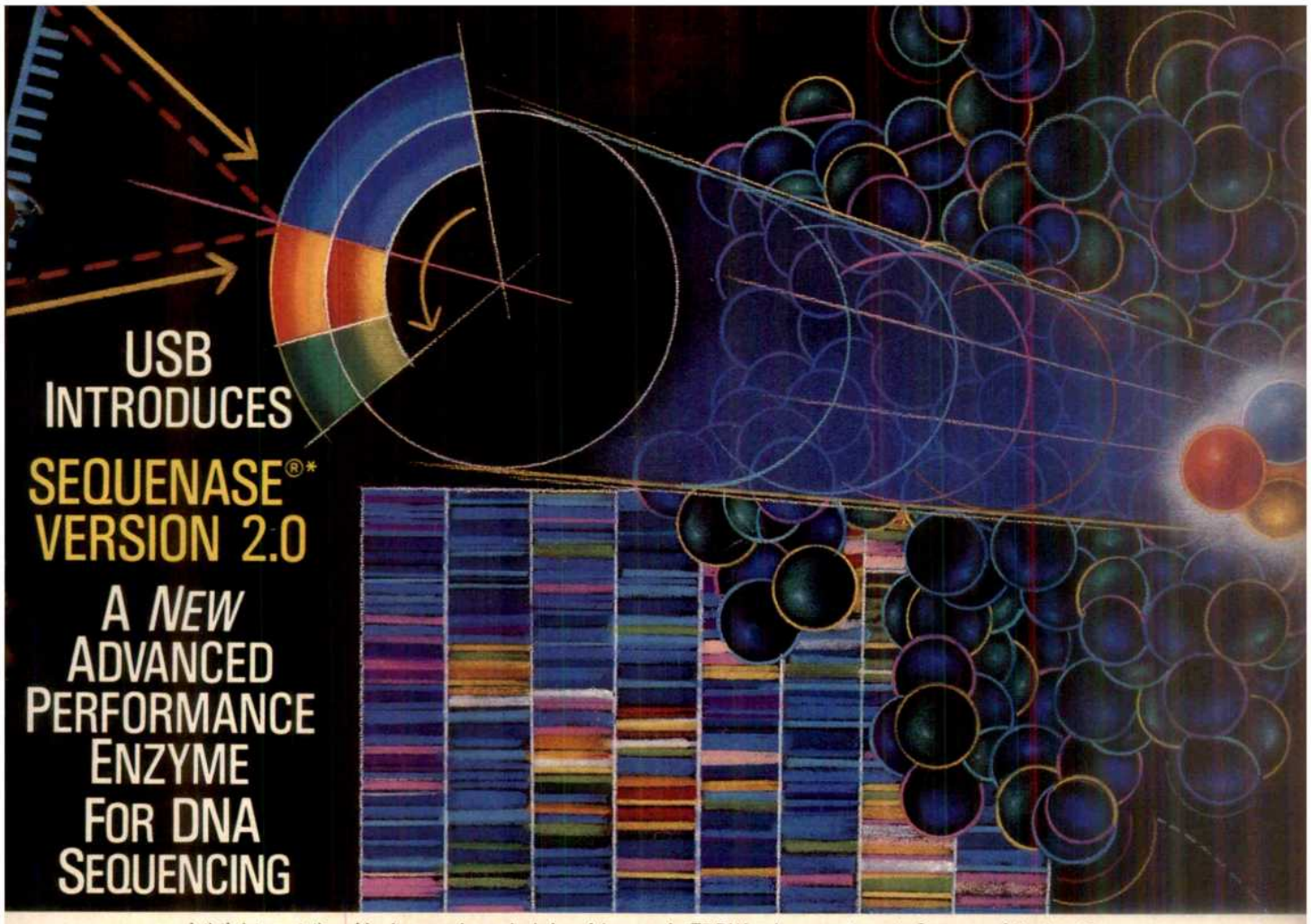
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An International Conference and Trade Exhibition is being organized in London jointly by the Royal Microscopical Society and the Electron Microscopy and Analysis Group of the Institute of Physics. It will see the coming together for this special occasion of the highly successful meetings run by the Institute of Physics (EMAG) and the Royal Microscopical Society (MICRO), with the Conference in the Institute of Education, London University, Bedford Way WC1 and the Trade Exhibition across Bedford Way in The Royal National Hotel.

This conference forms a part of "The Year of the Microscope" celebrating the first 150 years of the Royal Microscopical Society.

SCIENTIFIC PROGRAMME

Sessions

The conference will be divided into approximately 26 sessions in which original research presentations will be made. Each session will normally last half a day so that for most of the time four sessions will be in progress simultaneously. Normally two of these will be directed towards the biological and two towards the physical sciences.

Programme structure

Each session will comprise oral and poster presentations with the majority of contributed papers being presented as posters. Sessions may be preceded by Tutorial lectures and will normally have Invited speakers followed by a small number of selected talks from the Contributed papers.

Posters

Time will be allowed in the programme for poster viewing and in addition designated times will be timetabled when authors will be in attendance for discussion. Posters will be on display for the maximum time which can be programmed. Poster prizes will be awarded in several categories.

Publication

Short abstracts are required by 31 March. Abstract forms are available from the RMS Administrator at the address below. Authors will be notified regarding acceptance and mode of presentation in May and camera-ready manuscripts (four pages for contributed papers) are required by 13th September for publication in the two volumes (one Biological and one Physical) Conference Proceedings (I.O.P. Conference Series) to be published in December 1989. No distinction will be made in the published Proceedings between oral and poster presentations. Conference registration will include one volume of choice.

Invited speakers

The following speakers have agreed provisionally to present papers at the conference. So that coverage of rapidly advancing techniques will not be precluded, the programme will not be finalised until April 1989.

Dr. R.J. Hamers (Yorktown Heights, U.S.A.) "Developments in STM"
Prof. T.M. Jovin (Göttingen, W. Germany) "New aspects of fluorescent probes in biology"
Prof. D. Lansing-Taylor (Pittsburg, U.S.A.) "Multi-mode microscopy of cell dynamics"
Dr. J. Gillespie (Newcastle-upon-Tyne) "Single cell spectroscopy: A review"
Dr. J. Metcalfe (Cambridge) "Fluorescent molecular probes: A review"
Dr. M. Stewart (Cambridge) "Electron microscopy of frozen hydrated biological molecules"
Dr. P. Duncumb (Cambridge) "Superconductors: the role of electron microscopy and analysis"
Dr. C. Grovenor (Oxford) "Recent developments in atom probe microanalysis of materials with ultrafine microstructures"
Dr. L. Edelmann (Homburg/Saar) "Freeze substitution and low temperature embedding"
Dr. C. Chassard-Bouchaud (Parts) "SIMS in medicine and biology"
Dr. D.M. Shotton (Oxford) "Techniques of low light level imaging of Biological Specimens"
Dr. H.J. Tanke (Leiden, Netherlands) "Time-resolved fluorescence microscopy: Techniques and applications"
Dr. C.A. Warwick (New Jersey, U.S.A.) "Scanning cathodoluminescence of semiconducting materials"
Dr. E.D. Boyes (Delaware, U.S.A.) "Developments in low voltage SEM"

Prof. M. Rühle (Santa Barbara, U.S.A.) "HREM of ceramic/ceramic and metal/ceramic interface"
Prof. H. Hashimoto (Okayama, Japan) "High Resolution Microscopy of Dynamic Events"
Prof. A. Boyde (London) "Tandem scanning (confocal) microscopy and the third dimension"
Dr. A.J. Craven (Glasgow) "EELS techniques and applications"
Dr. R.S. Camplejohn (London) "Applications of flow cytometry"
Dr. P.J. Dobson (Oxford) "Reflection high energy diffraction and molecular beam epitaxy"
Dr. M. Hoppe (Wetzlar, W. Germany) "Problems solved by acoustic microscopy"
Prof. J. Kushibiki (Sendai, Japan) "Acoustic microspectroscopy"
Dr. D.A. Smith (Yorktown Heights, U.S.A.) "Interfaces and the microstructure of thin films"
Prof. J. Silcox (Cornell, U.S.A.) "Lithography"
Dr. L.P. Kok (Leiden, Netherlands) "Physics of microwave irradiation"
Dr. M. Boon (Leiden, Netherlands) "The application of microwave in pathology laboratories"
Mr. P. Jackson (Leeds) "Immunohistochemistry and the use of microwaves"
Dr. R.W. Horobin (Sheffield) "Overview of microwaves in morphological studies"
Prof. J. Venables (Sussex) "Auger reflection microscopy"
Dr. P. Kruit (Delft, Netherlands) "Coincidence methods in STEM"
Dr. N.C. Billingham (Sussex) "UV microscopy"
Dr. J.A. Reffner (Stamford, U.S.A.) "Molecular microscopical mapping with the FTIR microscope"

Contributed papers
Papers are invited on all aspects of microscopy and related techniques. They must contain new material not previously published. Authors can express preference for oral or poster presentation. With this in mind, selection of a contributed paper for oral presentation will be made, by the programme committee, in the interests of an attractive and coherent programme and complementing the invited talk(s).

Tutorial lectures
A number of tutorial lectures will take place throughout the Conference, preceding the appropriate session. These will be a feature of the meeting and are intended to introduce the interested non-expert to that field. The provisional list of tutorial lecturers and topics is as follows:

Prof. J.W. Steeds (Bristol) "Large angle CBED"
Dr. O.L. Krivanek (Pleasanton, C.A. U.S.A.) "Progress in parallel detection EELS'"
Dr. A. Dixon (Oxford) "Principles and applications of confocal fluorescence microscopy"
Dr. D. Haugland (Eugene, U.S.A.) "Fluorescent molecular probes in biology"
Prof. J.B. Pawley (Madison, U.S.A.) "The Micro World has three dimensions and sometimes four"
Dr. N.P. Carter (Cambridge) "Flow cytometry — What is it?"
Dr. P. Echlin (Cambridge) "Low temperature biological microscopy and analysis"

COMMERCIAL EXHIBITION

An exhibition of the latest microscopical instrumentation and all types of ancillary equipment will be held in the Royal National Hotel, just across the road from the location of the Conference. Admission to the exhibition is free of charge by Conference Badge or by Exhibition Only Badge and will provide delegates with an unrivalled opportunity to exchange views with the manufacturers on their range of products. Facilities for exhibitors wishing to give formal presentations, videos, etc. will be available.

Tickets for the Exhibition only are available from the RMS Administrator at the address below and from the registration desk once the Exhibition opens. The organisers acknowledge with gratitude the help and advice of the Trade Advisory Committee of the Royal Microscopical Society.

Abstract forms and further Information
Abstracts must be submitted on camera-ready forms in accordance with the instructions accompanying them. These can be obtained from the following address. The deadline for abstracts is 31 March 1989. An acknowledgement card will be sent by return post.

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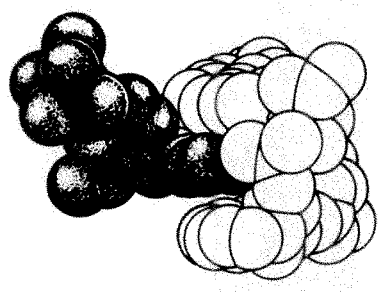
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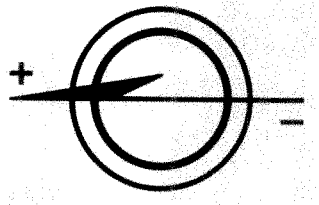
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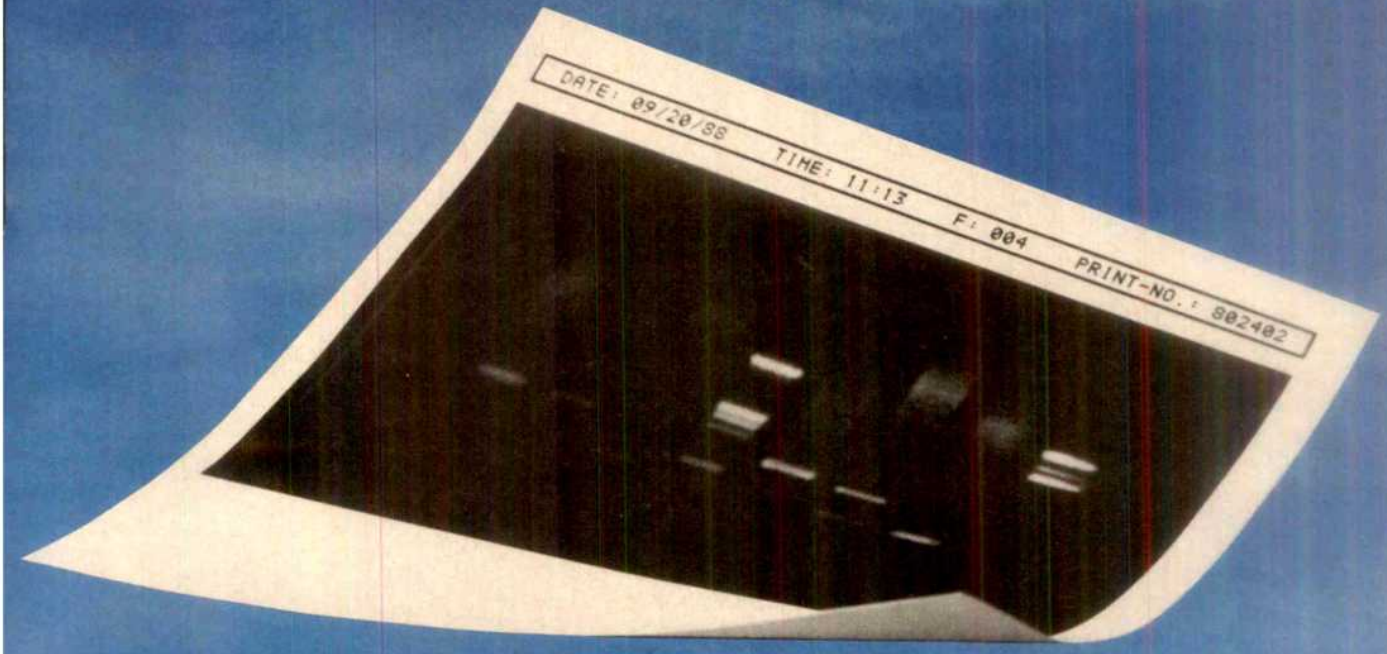
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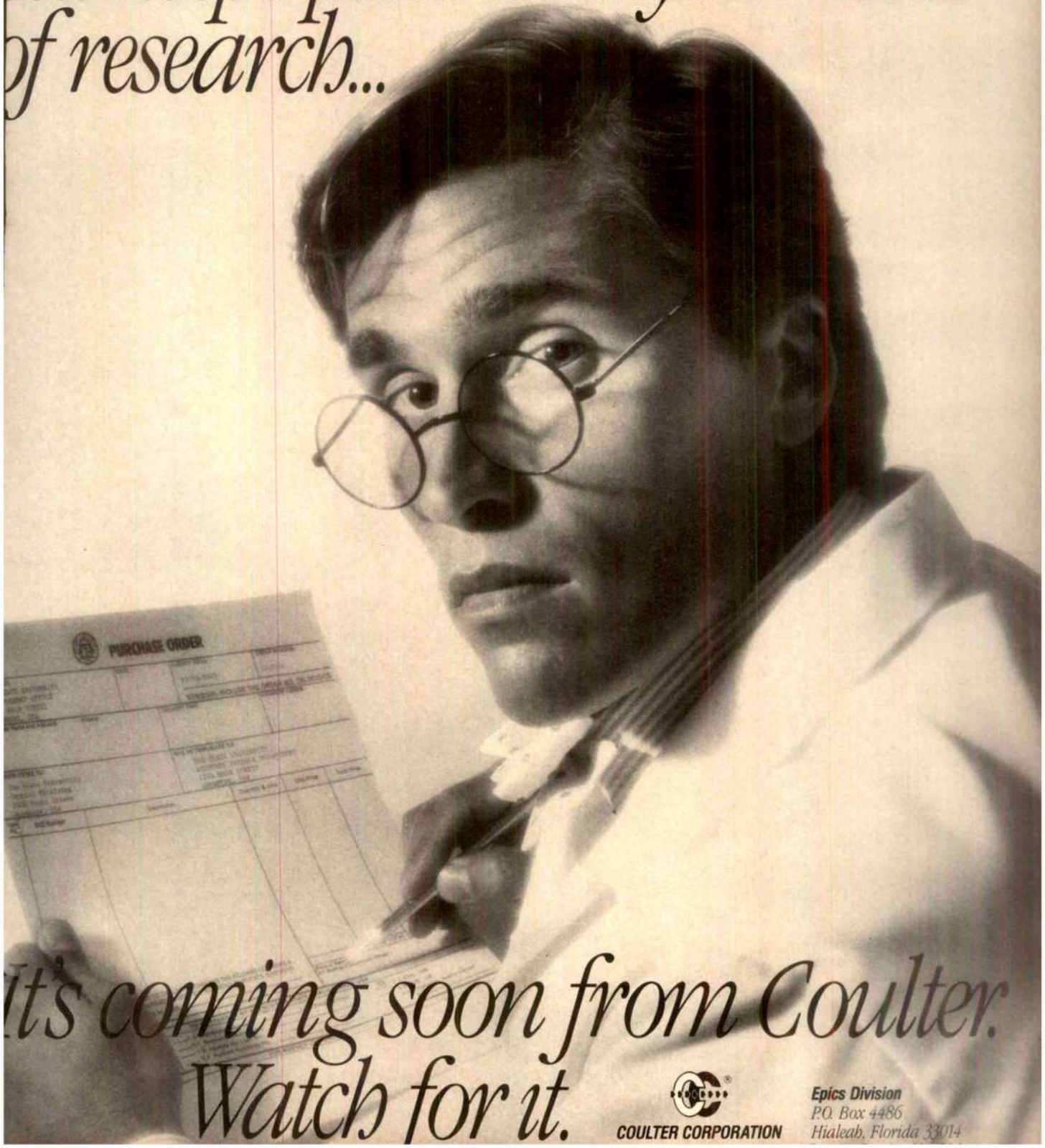
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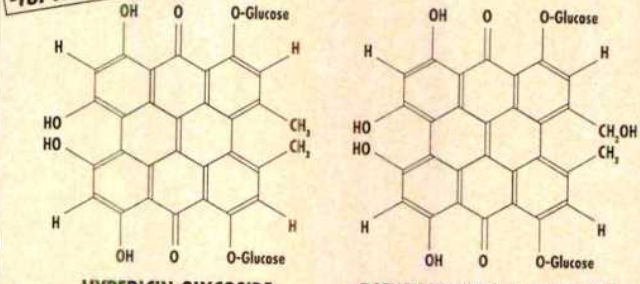
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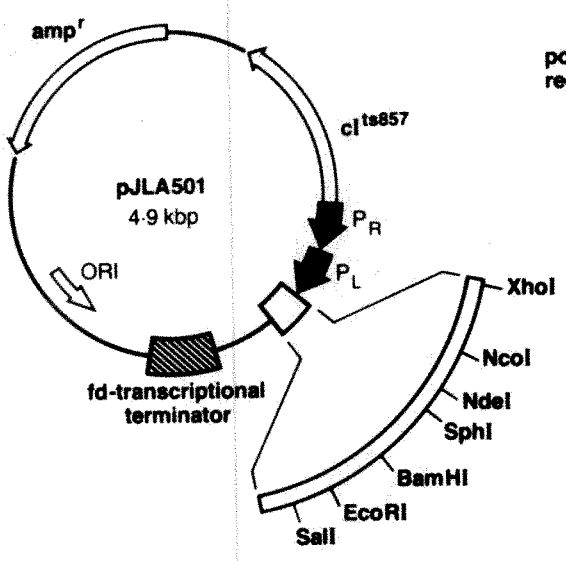
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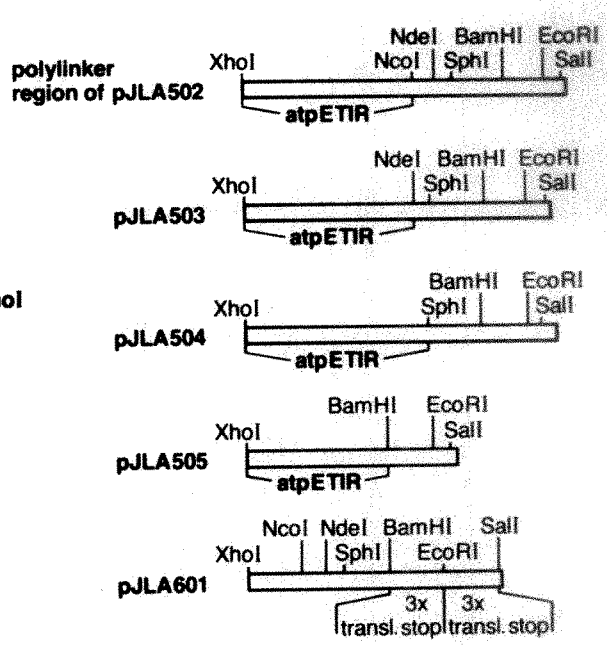
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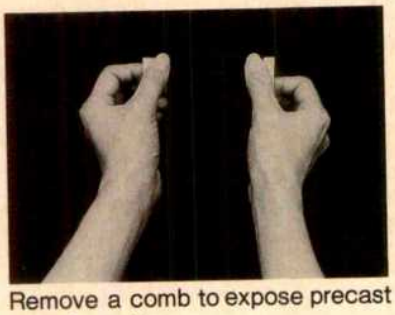


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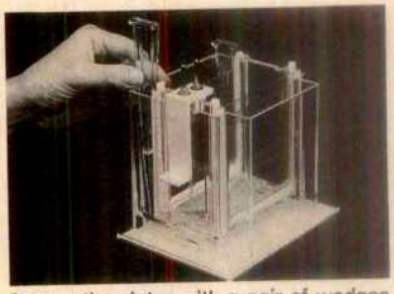
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Getting rid of poor people's debts

The US administration may have bungled its well-meant plan to lift the burden of indebtedness from the shoulders of the developing world.

WILL Mr Nicholas Brady, the new US Secretary of the Treasury, be the next victim of the Bush administration's unpreparedness? Last Friday, Brady stood up in Washington to outline a solution to the problem of the debts owed by the poorest countries in the world to the richer countries and their commercial banks. Going further than his predecessor, Mr James Baker (Secretary of State in the new administration), Brady argued for a reduction of the debts the poor countries cannot afford to repay. The details are still vague, but Brady urged that the World Bank and the International Monetary Fund (IMF) should have extra resources so as to be able to buy out the interests of the commercial banks in some of their outstanding loans.

But who now can lend more? Only Japan. To its credit, the government of Japan announced its approval of Brady's suggestions even before his speech had been delivered. It seems to be implicitly agreed that, in the outcome, Japan will exercise much greater influence in the international lending institutions, which Japan appears to welcome. The US administration is more cautious, saying only that the details of the Brady plan are still to be worked out. But Brady's speech will by itself set in train events that neither he nor the White House can control.

That something should be done, and urgently, is plain. The total indebtedness of the developing countries is estimated at $1.2 million million, some 20 per cent more than the annual federal budget of the United States. More than a third of the debt is owed by Latin American countries, most of whom cannot make the required repayments; the rest is scattered through the developing world, which is hardly in better shape. The way in which the prospects for development are crushed by this burden of debt is plain. The Bush administration deserves credit for recognizing more plainly than its predecessor that the debt imbalance is now the most serious threat to international stability.

So why not forgive the debt? Commercial banks in the West have been compelled by their auditors to accept that the loans they have made are unlikely ever to be fully repaid; during the past two years, they have written off roughly a third of the debts they are owed. But that realism does not in itself relieve developing countries of their debts, which remain nominally repayable in full. The banks set out to collect what they can from whom they can, pretending that the writing off of a third of the value of their loans is irrelevant in negotiations with individual borrowers. Without an agreement not yet reached between parties not yet identified, the banks have no choice.

But you cannot get water out of a stone, which is why the past year has been occupied with schemes for rescheduling developing country debt. The hope had been to stretch out debt and interest repayments over longer periods and, crucially, to lend the debtors extra cash to stimulate economic growth so that the attenuated debts would eventually be repaid in full. The first task has been relatively easy; the commercial banks lose nothing by rescheduling debts that would otherwise be repudiated. But extra cash has not materialized on a scale sufficient to allow the debtor countries to grow their way out of trouble. Instead, extra loans have often been barely enough to cover extra interest, and have been accompanied by economic constraints laid down by IMF which have often seemed intolerably onerous, whence the past month's disturbances in Caracas, the capital of Venezuela.

Brady's ambition is to short-circuit this process, which is admirable, and to do so within the context of a free market, which again makes sense. But how? This is where the plan tails off into thin air. In some form, funds provided by industrial countries (chiefly Japan) must be used to buy out the interests of the commercial banks in their doubtful loan agreements, presumably at a large discount. The banks would swap doubtful assets (their loans) for bonds whose face value is perhaps only a third as much, but which are somehow guaranteed by the international institutions. The obvious snag is that Brady's announcement gives debtor states an incentive immediately to diminish the book value of the loans they owe, while there can be no assurance that all the commercial banks owed money will take part in whatever scheme is eventually worked out.

It would have been better to put the horse before the cart, and to have negotiated the details of a workable scheme with those who will have to meet the costs (the commercial banks and the Japanese banking system) before giving other interested parties a chance prudently to escape their obligations. It is also plain that there can be no permanent settlement without an understanding of the extent to which the poor countries can expect to work off their debts by trading freely with their creditors. The

United States has a particular, but urgent, need to devise a coherent policy on relations with Latin America, shamefully neglected in the past eight years. But, now that Brady has spoken, all this has to be arranged by tomorrow or, at the latest, the day after tomorrow. This is a slipshod way in which to deal with such an important matter. □

All greens now

Politicians anxious about the environment seem no more ready than their predecessors to make prudent choices.

THE most obvious intellectual weakness of the environmental movement, already apparent in its first flowering in the late 1960s, is its habitual failure to tell what is important. So much is now clear in the general response to the otherwise welcome recognition by senior politicians that the greenhouse effect must be taken seriously. Suddenly, it seems, a whole range of public policy is legitimately to be dominated by what are called ecological imperatives. One result will be that public and private resources will be squandered needlessly. Another is that our capacity to deal with the important problems will be seriously impaired.

Here is a sample of how the wind is blowing. A few months ago, the government of the Netherlands decided to require that new motor cars should meet emission standards more stringent than those agreed by the European Community in a famous compromise reached only with the greatest difficulty four years ago — and to which the Dutch were then a party. The result (unless the decision is overturned by the European Court of Justice) may be that Dutch motor-cars will in future emit less pollution while the rest of Europe reverts to its older ways. In West Germany (see page 194), self-evidently innocuous experiments with genetically engineered plants must be carried out in a limbo that will be removed only when there is a legislation on the subject, likely to be the most restrictive in Europe. In Britain last week, a plan to build a railway link between the channel tunnel and the rest of Britain was hastily rerouted to meet environmental objections, at an estimated extra cost of £500 million. Mrs Margaret Thatcher, the British prime minister, is both consistent and right to say that the extra cost must be met by those who use the railway, which is unlikely to be commissioned until several years after the tunnel. But, on the Principle of the Conservation of Money, the extra £500 million spent to preserve the gentility of rural Kent will be £500 million less to spend on other worthwhile causes.

Collective sanity requires a better sense of proportion. All environmental protection in which governments are involved is at bottom a public purchase — one made on behalf of a whole community with resources drawn from it either by taxation or by the payment of user fees. The government of the Netherlands, for example, has implicitly arranged that the costs of reducing exhaust emissions will be met by Dutch motorists, who will pay more for their cars and more for their fuel. (The Netherlands may hope to recoup some of the extra cost if Dutch motor-manufacturers find they have an edge in the domestic market-place over their competitors.)

The British railway to the channel tunnel is in the same case. The preservation of a corridor through Kent has been judged worth £500 million, which the operators of the railway will have to find and then recover by means of a tax (extra fare) on the railway's users. Meeting the extra capital cost out of public funds (which the British government will be asked to do) would simply redistribute the burden.

Sadly, in all of these decisions, the communities on whose behalf governments have acted (or, in the case of West Germany, failed to act) have not been presented with a choice. Yet the extra cost of the British railway far exceeds what would be the cost of drawing up and enforcing legislation to make British farm foodstuffs free from Salmonella infection but may be a more prudent investment. So should not the governments committing these substantial sums of money ostensibly in the public interest ask the communities they are supposed to serve what choice they should make between the alternatives? Surprisingly, even the British government, which likes to think of itself as strong, seems to prefer to be pushed about in one direction or another as local or otherwise special interests make themselves heard.

The conversion of people such as Thatcher to a proper concern about the greenhouse effect should have engendered a more resolute view of environmental costs, but has not. Yet the crude arithmetic of the case is as accessible as the back of the nearest envelope. Suppose there is eventually a carbon dioxide convention, and that countries such as Britain have to generate all their electricity from fuels other than hydrocarbons. That could entail building 50 million kW of nuclear generating capacity over say 20 years, which would work out at an investment of £5,000 million a year if the extra cost of nuclear generating capacity is merely £2,000 a kilowatt. That simple arithmetic does not imply that nuclear capacity is the only alternative, but unconventional sources of electricity would probably on present form be more expensive, nor does it allow for the replacement of fossil fuels in other energy-consuming machinery, vehicles for example.

In short, if it should eventually be necessary to reduce carbon dioxide emissions, the costs are likely to be orders of magnitude greater even than the sums now being frittered away on the railway to the channel tunnel. One way of responding to that dilemma is to say that if measures to combat the greenhouse effect are likely to cost several thousand million pounds a year, £500 million or so for Kent is mere chicken-feed. But that is also the route to national impoverishment. The more prudent course is to require than people should choose. Thatcher, above all politicians of her persuasion, should be eager to remind people that that is not merely their right but their duty. □

Changes in misconduct investigation planned

- Present procedures declared inadequate
- New legislation may obstruct plans

Washington

THE US Public Health Service (PHS) has decided to create a new mechanism for dealing with scientific misconduct by establishing an Office of Scientific Integrity in the office of the director of the National Institutes of Health (NIH).

This move comes after months of internal debate on how best to tackle the growing concern that current procedures for investigating alleged scientific misconduct are inadequate. But the rapid implementation of the new plans may not be easy, for they seem to be at odds with legislation about to be introduced in Congress.

Virtually nobody is satisfied with present procedures for dealing with scientific misconduct. Investigations have taken much longer than expected, there have been squabbles over who should investigate cases and Congress has felt that NIH may lack the will to tackle the difficult issues that inevitably arise. A report from the Institute of Medicine urges that encouraging proper research conduct is an important part of preventing misconduct (see ***Nature* 337, 588;** 16 February 1989). Accordingly, the new office at NIH will be responsible not only for investigating alleged misconduct but also for promoting high standards of laboratory practice.



The new office will make recommendations to the Assistant Secretary for Health, who is the head of PHS. The plan also calls for an Office of Scientific Integrity Review in the office of the assistant secretary to oversee investigations by the NIH office and to help develop policies for dealing with issues of integrity and misconduct.

Although the new office of scientific integrity will be located in the office of the NIH director, it will be responsible for investigations and procedures for all PHS agencies, including the Alcohol, Drug Abuse and Mental Health Administration and the Centers for Disease Control.

Congress has put pressure on PHS to make changes in the way it deals with misconduct. Last year, there were several hearings that drew attention to problems in current procedures. Earlier this year, Representative Henry Waxman (Democrat, California) asked the Inspector General of the Department of Health and Human Services to determine whether PHS was putting sufficient resources into its investigations.

Legislation is at present being drafted by Waxman and Representative John Dingell (Democrat, Michigan) — head of the powerful House of Representatives Energy and Commerce Committee — that would also create an office of scientific integrity, but place it in the assistant secretary's office, not at NIH. It seems certain that Congress will require assurance of a serious commitment to investigating misconduct before it agrees to let NIH retain primary responsibility for that role.

Joseph Palca

US SCIENCE POLICY

Who should decide policy?

Washington

THERE is a need for a new government body to set national goals in science and technology in the United States. That much was agreed at hearings held over the past two weeks by the House of Representatives Subcommittee on Science, Research and Technology and the Senate Committee on the Budget. But the form that the new policy-making body should take was a matter of great debate.

The consensus that the United States lacks a coherent national policy in science and technology has arisen for two reasons. In recent years, emerging big projects, such as the sequencing of the human genome and the study of global climate change, have cut across government agency boundaries. But there is no central government body to determine the relative importance of these projects, and the budgets they should command. On another front, Congress and industry are realizing that the United States is being left behind in development of key new technology, such as high-definition television (HDTV); that is also perceived as due to a lack of a guiding national policy.

Former presidential science adviser George Keyworth revived the idea of a new department at the House hearing. But several subcommittee members and witnesses dismissed this idea. William Carey, former executive director of the American Association for the Advancement of Science and a former assistant director of the Bureau of the Budget, says a new department would be "crippled" by lack of support from other departments and agencies. And when Clyde Prestowitz of the Carnegie Endowment made a similar suggestion at the Senate hearing, Senator Ernest Hollings (Democrat, South Carolina) said "We would all be dead and gone" before such a department is realized.

Carey instead favours proposals recently put forward by the National Academy of Sciences which call for collaboration between the Office of Management and Budget and the president's science adviser in determining priorities, objectives and budgets of large projects. A step in that direction has already been taken by the Committee on Earth Sciences of the Federal Coordinating Council for Science, Engineering and Technology, which has for the first time prepared a cross-agency budget for research on global climate change in 1989 and 1990.

Under the academy proposal, cross-cutting research activities would be reviewed by budget and appropriations committees in Congress before being broken down for consideration by individual agencies. Another approach, put forward by Lawson Crowe (University of Colorado), is to establish a national commission, composed largely of scientists, that would rank large-scale projects according to scientific merit, social benefit and "programmatic implications".

But most subcommittee members seemed more concerned with mechanisms for strengthening economic competitiveness than with scientific policy. Almost every witness was asked what should be done about HDTV, which is fast becoming the touchstone in Congress of the competitiveness of US industry.

The hearings are almost certainly a harbinger of a year of debate in Congress over the question of how to set science and technology policy. The same issues are certain to come up again when President George Bush's plans for giving his science adviser the status of an assistant to the president and for appointing a panel of independent advisers are put forward as legislation.

David Swinbanks

NUCLEAR POWER

Industry struggles on despite opposition

Washington

WILL nuclear power play a role in the future energy plans of the United States? The Nuclear Power Oversight Committee, a nuclear power industry body, issued a white paper (policy document) last week calling for an easing of government regulations so to encourage the building of new nuclear plants. But industry analysts say nuclear power is dying in the United States, not because of regulation but because the industry has lost economic and political viability.

James O'Connor, chairman of the committee, says that growth in demand for electricity in recent years has far exceeded even the power industry's projections and that the United States is now running "perilously close to capacity margins that leave very little room for error". He points to the emergency measures, such as voltage reduction, that many utility companies had to take during last summer's heat wave as a sign of the serious supply difficulties that lie ahead.

If demand for electricity in the United States continues to grow at 2–3 per cent a year, 120–220 large new nuclear power plants will have to be built by the turn of the century. The white paper argues that new plants could offer the most cost-effective new source if the government were to ease regulatory controls by, for example, introducing a single-licence system instead of, as now, requiring utility companies to obtain both a construction and an operating licence for new plants.

But Christopher Flavin, vice-president for research at the Worldwatch Institute, who has been monitoring the nuclear industry for the past decade, says the industry's figures for the cost of nuclear power are based on existing plants both old and new. Since the Three Mile Island accident, increased safety requirements have quadrupled plant construction costs and nuclear power is now more than "twice as expensive as competing sources". But O'Connor argues that the biggest additional cost in building new plants is delay due to complex regulatory controls.

Concern about global climate change has prompted Congress to reassess the pros and cons of nuclear power, an energy source free from carbon dioxide emissions, as the industry is ready to point out. But Flavin says that, if the new legislation introduced by Congress to deal with global warming is accepted, there will be a "stampede" towards the development of renewable energy sources, not nuclear power.

Public opposition to nuclear power in the United States remains widespread. Legislators introducing global warming bills have attentuated or omitted references to nuclear power to gain wider acceptance.

David Swinbanks

Legacy of Three Mile Island

Boston

A DECADE after the partial meltdown of the Three Mile Island (TMI) Unit 2 reactor near Harrisburg, Pennsylvania, on 28 March 1979, cleaning up continues at a cost of $1,000 million — nearly $300 million more than the cost of building the reactor in the first place. But the implications of the accident for the commercial nuclear industry are almost certainly far greater.

So far, workers have removed most of the damaged nuclear fuel from the Unit 2 reactor vessel. This has been accomplished by a team of more than 1,000 people, including a group operating specially designed equipment from a rotating platform above the reactor vessel. Working almost continuously since October 1985, the clean-up team has removed radioactive debris ranging in size from gravel-like material to a fused hard crust, up to 5 feet thick, formed from the molten fuel rods during the accident.

If all goes according to plan, the 'defuelling' process should be complete by the end of 1990, when Unit 2 will be shut and monitored for approximately 30 years, until the end of the planned lifespan of the operating Unit 1 reactor nearby. A full decommissioning and dismantling of Unit 2 will be postponed until the job can be undertaken for both reactor units together, according to Doug Bedell, spokesman for GPU Nuclear, TMI's owner.

Bedell emphasizes that enough material has been removed to dispel any chance of "a critical event", but he acknowledges that, because residual caesium has permeated the basement walls, "unacceptable" and potentially dangerous levels of radiation will still be left in the basement area of the Unit 2 reactor until decommissioning begins next century.

Since the clean-up began, 150 tons of highly radioactive debris has been hauled away from the reactor. But because there is not yet a functioning repository for high-level commercial nuclear waste anywhere in the United States, the debris from TMI continues to be stored "temporarily" at the National Engineering Laboratory in Idaho where researchers have studied the material to reconstruct the precise course of the accident. A plan to evaporate the remaining 2 million gallons of radioactive water at the TMI site still awaits approval from the Nuclear Regulatory Commission.

The accident at TMI Unit 2 began after only 11 months of operation when a pump providing feedwater to the reactor core clogged during full power operation. After the plant shut down automatically, a relief valve opened to diminish the built-up pressure and temperature in the reactor, letting out steam and water.

Undetected by the operators, this valve remained open for 2 hours, allowing the escape of nearly a million gallons of the coolant water covering the reactor fuel. As approximately half of the reactor core became uncovered, the radioactive water overflowed designed catchments, collecting in the basements of the reactor and auxiliary buildings. Without the coolant water, temperatures in the pressurized-water reactor rose quickly, causing some of the uranium fuel to melt.

The accident at TMI prompted a widespread response in the nuclear industry. Within weeks of the disaster, the Electric Power Research Institute (supported by the utilities) established an organization called the Nuclear Safety Analysis Center (NSAC) to analyse the accident and to inform other utilities of any future malfunctions or other information that could be useful.

Later that year, the industry also formed the safety-oriented group called the Institute of Nuclear Power Operations (INPO). Among its other functions, INPO runs a computer network linking nuclear power plant operators with experts at INPO and NSAC to provide instantaneous advice and information. According to William Layman, since the accident at TMI, the industry has learned that "the price of safety is eternal vigilance".

But although the industry can point to strengthened safety procedure in the aftermath of the TMI accident, most agree that the incident's greatest legacy is a sustained level of lingering public fear and opposition to nuclear power in the United States.

More than 2,000 lawsuits claiming health and psychological damage from the TMI accident are still pending, despite measurements after the accident that showed that the highest possible whole-body dose to any one individual was less than 100 millirem, or only slightly greater than the average most people are exposed to from medical and dental radiation in a year.

Most observers agree that the TMI accident was a clear turning-point for the US nuclear industry, although poor performance, steeply rising construction costs and slowly growing demand for electricity may earlier have presaged the industry's decline. Although performance has improved and dependence on nuclear power as a fraction of energy supply in the United States has since grown, no plants have been ordered since the accident and all of the 47 units ordered in the United States since 1974 have been cancelled.

Seth Shulman

VETERINARY SCIENCE

Plan to close schools resisted

London

THE review of veterinary science at British universities, published earlier this year, has been so fiercely attacked that it seems unlikely that its proposals will be implemented unchanged. The review (see *Nature* **337**, 294; 26 January 1989) recommended that the veterinary schools at Glasgow and Cambridge should be closed.

Representatives of the veterinary profession now say that the closures will increase manpower shortages, lead to a lowering of standards in the veterinary profession and persuade more British researchers to go to the United States to work. In the universities, even those who agree that some reduction of capacity would be wise say the working party has picked the wrong targets.

The review is one of a series being carried out for the University Grants Committee in an attempt to decide how to use most efficiently the limited financial resources of Britain's universities. The working party for the veterinary sciences, whose chairman is Sir Ralph Riley, was told that a maximum intake of 335 students a year will be necessary to meet future manpower requirements and was asked to frame its recommendations accordingly.

To ensure that clinical sciences are taught with satisfactory spread and depth, the working party concluded that each school should have at least 36 clinical teachers. With some redistribution of posts, four schools, instead of the present six, would be sufficient. In order that the remaining schools are as widely spaced geographically as possible, the working party concluded that one of the two schools in Scotland and one in the South-East of England would have to be closed down.

Because there is room for expansion at Edinburgh, the Glasgow school was selected for closure. But the review says that the Edinburgh school should cease to exist in name, and that a new Scottish school would be formed by a merger of the two.

The Royal College of Veterinary Surgeons and the British Veterinary Association last week launched a furious attack on these proposals. They argue that future manpower needs require an intake of 400–500 students a year, which four schools could not provide. The registrar of the college, Alistair Porter, says the shortage of veterinary surgeons has already brought an influx of manpower from other countries.

The president of the Royal College, John Parsons, says that researchers would prefer to move to the United States rather than agree to be relocated in Britain. Since the announcement of the proposed closures in January, job offers have started coming in to researchers at Glasgow and Cambridge.

But those at the highly regarded department of veterinary pathology at the University of Glasgow are unlikely to be lured away. Even if the Glasgow school does move, there are provisions in the Riley report for this department to continue to exist at the University of Glasgow. The campaign against closure of the veterinary school in Glasgow has won widespread support in the local community: a petition with more than 300,000 signatures is to be sent to the prime minister.

Christine McGourty

SCIENCE JOBS

Students hang back, researchers may quit

Sydney

THE poor job market for Australian scientists is apparently starting to cause problems for the future of Australian science. The signs of trouble are clear from the figures for university enrolment for this academic year. According to a report by Australian Vice-Chancellors Committee, universities have had to lower entry standards to find enough science students. At the University of Sydney, entry scores in the science faculty dropped from 320 out of a possible 500 in 1987 to 300 this year, the lowest entry aggregate for any faculty. The University of Melbourne was forced to drop entry requirements from 295 in 1988 to 279 this year in order to fill its quota of science students.

But even those already on the career ladder are finding the climb tough. Seventy-five per cent of non-tenured Commonwealth Scientific and Industrial Research Organisation (CSIRO) scientists are unhappy with their career prospects, according to a survey by the recently formed Australian Science Action group, headed by Dr Greg Tanner, a CSIRO plant scientist. Forty per cent of the total sample size of 175 say they are prepared to leave Australia. Of these, 28 per cent indicated that they would pursue science overseas, while the rest say they would leave science altogether.

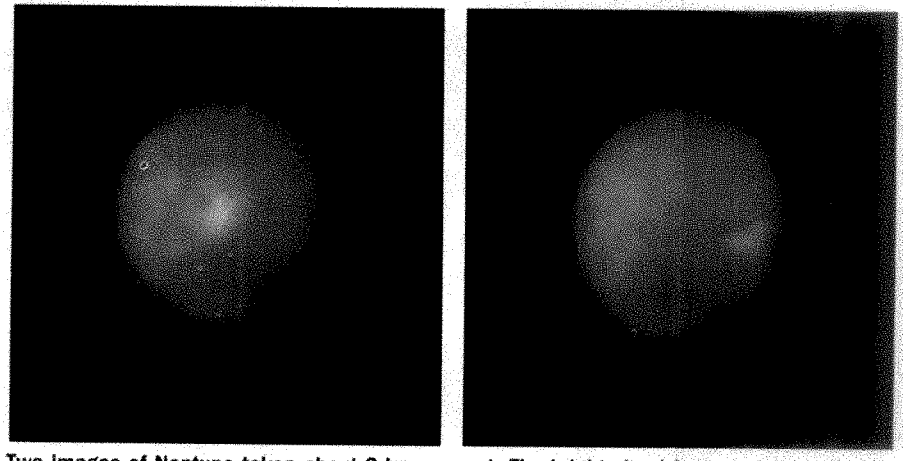
The CSIRO study shows that disillusion is greatest among younger postdoctoral researchers without tenure. The disaffection is particularly striking among biologists and chemists; those in the physical sciences less commonly regarded their prospects as poor. Women scientists with families are also discouraged about staying in science because of the reduced number of part-time positions available.

Tania Ewing

NEPTUNE FLY-BY

Voyager's first glimpse of distant planet raises NASA's hopes

PROSPECTS for Voyager 2's fly-by of Neptune on 24 August are encouraging, according to the mission controllers at the Jet Propulsion Laboratory in Pasadena. The colour images shown here, taken on 23 January when Voyager was about 185 million miles (309 million km) from the planet, already show detail down to 3,500 miles (6,000 km), better than that available from the best telescope images from Earth. The fact that distinct cloud features are visible when the spacecraft is still so distant suggests that images obtained during August will show far more detail than was visible in the atmosphere of Uranus, which Voyager 2 encountered in January 1986. The natural colour of Neptune, like that of Uranus, is a pale blue-green, caused by methane gas in the atmosphere, which absorbs red light. The images here were obtained through violet, clear and orange filters. □



Two images of Neptune taken about 2 hours apart. The bright cloud feature visible near the centre of Neptune's disk in the earlier (left-hand) image has moved to the right edge of the planet in the later image. This is in line with the 17- to 18-hour rotation calculated from Earth-based observations. A dark band of clouds, similar to structures seen on Jupiter, Saturn and Uranus, can be seen encircling the southern pole. □

PLAGIARISM DISPUTE

Clinical researchers at odds

Washington

A FEDERAL appeals court has written the latest chapter in a bitter dispute over plagiarism between a former faculty member of Albert Einstein College of Medicine in New York and her mentor, now vice-chairman of nuclear medicine at the medical school. A three-judge panel overturned a lower court's ruling, and declared that Leonard Freeman violated copyright law when he put his name on a document prepared by Heidi Weissmann, but Freeman's attorneys have appealed against the latest decision. The case threatens to drag on.

Told one way, there seems little doubt that Freeman misappropriated Weissmann's work. In August 1987, Freeman was invited to present work on liver imaging in a review course at Mount Sinai School of Medicine in New York. Freeman took a syllabus prepared by Weissmann for a similar course sponsored by the Radiological Society of North America in 1985, deleted Weissmann's name from the manuscript and put his in its place, and changed the title from "Hepatobiliary Imaging" to "Gastrointestinal Nuclear Medicine Hepatobiliary Imaging". The same syllabus appeared in a book published in Taiwan under Freeman's name after a symposium in December 1986 in the Republic of China.

But the case can also be read differently. Weissmann and Freeman began their association when she was a resident at Einstein in 1977, and had collaborated on liver-imaging work since 1980. After finishing her residence, Weissmann became a faculty member at Einstein, working in Freeman's division.

The 1985 syllabus used by Weissmann was largely drawn from previous versions of the syllabus which were jointly produced, but which differed in some references, illustrations and some new text. Furthermore, when Weissmann filed her suit to prevent Freeman from presenting the syllabus at Mount Sinai, she was in the midst of a lawsuit accusing Einstein of sexual discrimination over issues of salary and sabbatical leave.

A district court judge in New York agreed with Freeman's contention that the syllabus was a product of collaborative research, and a continually evolving 'stock' piece to be used when either was called upon to teach a course in hepatobiliary imaging techniques. The judge ruled that Freeman should be considered a joint author of the syllabus, and therefore could not be guilty of violating copyright of it. He also ruled that Weissmann's modifications to the 1985 syllabus were insufficient to support separate copyright protection and that, even if Freeman was not considered a joint author of the syllabus, his intended use of the document fell under the 'fair use' doctrine as it was intended for teaching and not personal profit.

But the appeals court saw things differently. In a 2–1 decision, the court ruled that Freeman's use of the syllabus was a violation of copyright. The majority opinion states that the decision of the lower court "stands copyright law on its head" by concluding that joint authorship of prior work "automatically makes the two joint authors co-owners of the derivative work". The court goes on to state that originality is rightly prized in law, and alludes to Dostoevsky's example in *The Idiot* that a person's worth in revolutionary Russia "did not depend so much on money or position as it did on one's originality". The appeals court also ruled that, although he did not stand to gain financially from the copyright infringement, Freeman did gain in stature professionally by passing Weissmann's work off as his own.

Einstein and Montefiore Medical Center, where both worked, have been firmly behind Freeman throughout the dispute. Part of Freeman's legal costs have been met by his employer. An open letter from Montefiore's president Spencer Foreman following the lower court's ruling last May declared that the "Federal District Court has completely vindicated our colleague Leonard M.Freeman", and refers to Weissmann as "a former Montefiore employee and former student", rather than as a former faculty member. The letter ends with "I know you are all as pleased as I am with the decision reached by the federal court".

Weissmann is now out of work. The medical school maintains that she resigned when she failed to return to work after a sabbatical which ended in September 1987. Weissmann says she was fired because when she tried to go to her office in August of that year, days after taking legal steps to prevent Freeman from handing out the syllabus at Mount Sinai, hospital security guards confronted her and demanded that she hand over her office keys. Freeman, meanwhile, has been promoted to vice-chairman of his department.

Freeman says the dispute should never have gone to court.Weissmann says she by-passed university mechanisms for resolving disputes in the copyright case because she did not think the university would give her case adequate attention.

Einstein has had a poor track record in its attitude towards female faculty. It has been the object of a class action suit filed in the 1970s alleging sexual discrimination on salaries that has still not been settled. Eugene Girden, a lawyer for Freeman, says it is ironical that Weissmann is suing Freeman, as he was one of her strongest supporters at the university.

Weissmann's case has been taken up by the National Coalition for Universities in the Public Interest, a Washington watchdog group, which intends to file new charges in Weissmann's discrimination suit still pending in New York State court. Leonard Minsky, the coalition's executive director, argues that Weissmann's experience is just one of a series of cases involving plagiarism, sexual discrimination and conflict of interest in US universities.

Joseph Palca

MENTAL HEALTH

Sterilization protests

Munich

A PROPOSED new law that would make it legal to sterilize people judged mentally incompetent without their consent has prompted a vigorous protest in West Germany from those who fear the law might some day be used to justify enforced sterilizations of a broader class of people.

The provisions on sterilization are part of an otherwise welcome reform of the rights of mentally disabled people. The legislation would ban the sterilization of minors, and has been well received on that account across the political spectrum, especially because of its emphasis on counselling and support for people unable to speak for themselves. But critics say that the legislation also contains a potentially dangerous loophole that might at some future stage be widened by allowing the sterilization of adults who are mentally incompetent.

Hitherto, up to 1,000 sterilizations a year have been carried out in West Germany on people — minors and adults — deemed legally incapable of handling their own affairs. Many of these sterilizations are done at the request of parents of mentally handicapped children. Under West German law, incapacitated people have had no legal recourse against the procedure.

The new legislation permits the sterilization of people who do not specifically object in cases where pregnancy would create an "emergency situation". Any objection on the part of the person in question would result in the cancelling of the sterilization.

The Green Party, a judges' organization and handicapped rights groups have protested against the law and are preparing to fight it in parliament. The current law, which dates back to 1900, deprives mentally incompetent adults of all rights, leaving them the equivalent of a child in legal terms. The new law was meant to be a landmark in a series of social programmes that have been staggering through parliament in recent months. The proposal has been sent to both houses of the West German parliament for discussion. The government hopes to gain approval for the law by the end of this year. Steven Dickman

LAKE BAIKAL

Invitation from Soviet centre

London

PLANS to found an international research centre on Lake Baikal in south-east Siberia have been formally agreed in the Soviet Union, where the Soviet Academy of Sciences has deputed responsibility for the project to its Siberian Division. The division is now inviting putative members of the centre to declare their interest, and plans to hold a constitutional conference at Irkutsk in the second half of this year.

The formal announcement, contained in a cable to the Editor of *Nature*, is as follows: The Praesidium of the Siberian Division of the Academy of Sciences of the USSR announces its intention of organizing the Baikal Centre for Fundamental Ecological Research — an organization open for the collaboration of Soviet scientists and scientists of all other countries in studies of the greatest ancient freshwater lake, Lake Baikal.

The facilities of this centre are sited, and will be further developed, at Listvyanka, on the shore of Baikal, and in Irkutsk, 70 km away. The Limnological Institute at Irkutsk will provide research vessels (six ships), laboratory rooms, expeditions, equipment, hotel rooms, etc., as agreed with participating groups and contracted for.

It is proposed that the scientific programme of the centre should include the following topics:

- Interdisciplinary studies of the Baikal ecosystem using the techniques of hydrodynamics, hydrochemistry, hydrobiology, remote sensing and mathematics in order to create a conceptual working model of this ecosystem.
- Studies of the speciation of the unique Baikal endemic complex of some 1,500 organisms and of the evolution of their nucleic acids and proteins.
- Investigation of the global transfer of elements and priority pollutants.
- The development of methods of environmental monitoring.
- Geological studies of Lake Baikal and of the history of its formation.

The Baikal International Centre will be operated by an international board of directors appointed by the founding parties. These will include the Siberian Division of the Academy of Sciences of the USSR, other Soviet organizations and organizations from other countries which agree to support the centre financially and to participate in its activities.

The board of directors will consider proposals for research projects and will select those to be carried out in each succeeding year on the basis of the reports of international referees. It will also decide what proportion of the budget will be devoted to each project selected in advance of each year's research.

Potential founding members of the Baikal International Centre — scientific institutes, scientific societies and international agencies — are hereby invited to open discussions with the Limnological Institute to clarify the framework of the agreement with the Siberian Division regarding participation in and support for the activity of the centre. Please write or cable to Professor M. A. Grachev, Lermontova 281, 664033 Irkutsk, USSR.

Inquirers will be supplied at their request with a draft charter of the centre, a list of the scientists who have already endorsed the concept of the centre and who have participated in collaborative research at Baikal as well as with any other relevant information.

It is hoped to hold a conference with representatives of the founding members of the centre at Irkutsk in the second half of 1989.

The cable is signed "Praesidium of the Siberian Division of the Academy of Sciences" and has been verified by telephone in a conversation with Professor Grachev. A general account of the Siberian Division's plans will be found in *Nature* **337**, 111; 12 January 1989. □

■ See page 209 of this issue for Professor Grachev's account of the canine distemper virus infection of Lake Baikal seals.

US UNIVERSITIES

MIT studies relations with Lincoln Laboratory

Boston

THE Massachusetts Institute of Technology (MIT) has announced the formation of a committee to investigate the university's relationship with the federally sponsored Lincoln Laboratory in Lexington, Massachusetts. The laboratory's $386-million annual budget is paid by the United States government, but the institute operates the facility.

At the core of the inquiry is the laboratory's emphasis on defence-related research involving radar and advanced electronics. Much of the work at the laboratory is classified, despite a university policy that eschews classified research on campus.

Paul Gray, president of MIT, says the new committee, headed by Joel Moses, chairman of MIT's electrical engineering and computer science department, will "explore alternative methods" for administering the Lincoln Laboratory. Gray has asked the committee to report by September. The group's twelve members also include Jerome B. Weisner, former president of MIT, Francis E. Low, former MIT provost, and Walker Morrow, Jr, director of Lincoln Laboratory. Seth Shulman

NEWS IN BRIEF

Third shuttle launch

Washington

THE third space shuttle launch since flights resumed took place last Monday (13 March) when the shuttle Discovery lifted off from Cape Canaveral in Florida. The lift-off was delayed one hour and forty minutes by poor weather conditions at the launch site. The mission's primary goal is to deploy a third tracking and data relay satellite that will complete a system of communications satellites in geosynchronous orbit that facilitate much higher data rates from spacecraft to ground stations. The new system is essential to make optimal use of data from the Hubble Space Telescope to be launched in December. This telescope was costly and expectations of it are high (see page 199).

The next shuttle mission, scheduled for 28 April on the shuttle Atlantis, will launch the Magellan spacecraft, a planetary probe bound for Venus. J.P.

Blaser to retire from PSI

Munich

J. P. BLASER will be retiring in the spring of 1990 as director of the Swiss Paul Scherrer Institute (PSI) for nuclear research in Villigen, near Zurich. The institute is currently seeking a replacement. Blaser, 66, was formerly director at the Swiss Institute for Nuclear Research, which was fused with the Federal Institute for Reactor Research at the end of 1987 to form PSI. S.D.

New authority in view

London

The task of reaching international consensus on how to combat global warming was tackled last week at an international conference on the environment held in the Hague, and organized by the governments of France, the Netherlands and Norway.

The declaration at the end of the conference calls for a new authority to be set up in the United Nations to be responsible for combating global warming. It says that the authority should undertake or commission scientific research and ensure the distribution of information between nations, including the sharing of technology. The authority would set standards for protection of the atmosphere and monitor compliance. But the conference decided not to endorse the imposition of economic sanctions on offending nations; measures to promote compliance would be referred to the International Court of Justice.

It was agreed, however, that developing countries should be compensated for any burden placed on them by the regulations, according to their responsibility for pollution. The organizers were criticized for their selective invitations — the United States and the Soviet Union were among those not invited. But the Dutch say all nations were invited to sign the declaration. C.McG.

REMOTE SENSING

SPOT on, but Landsats off?

Paris & Washington

US and French remote-sensing satellites are facing very different futures. EOSAT, the Earth Observation Satellite Company that operates the two orbiting Landsat satellites for the National Oceanic and Atmospheric Administration (NOAA), has been instructed to begin shutdown procedures for both satellites, putting them in a stand-by mode from the end of this month. By contrast, SPOT, the French Earth-observing satellite, had its third anniversary in space marked by a book about its history*.

The chief reason for cutting down the Landsat programme is money. Both Landsats 4 and 5 have exceeded their design lives, and although they are still providing useful information, neither the White House nor Congress has been interested in spending money on upgrading them.

NOAA is obliged to provide EOSAT with money to operate the two satellites, but with with its own budget thinly stretched, the agency has been looking to EOSAT revenues to help defray expenses. EOSAT has responded by arguing that current Landsat revenues are needed to prepare for Landsat 6, now due to be launched in 1991.

EOSAT maintains that $9.4 million would allow it to keep operating the two Landsats until the end of the fiscal year (at the end of September). Although the details of a package have not been worked out, NOAA assistant administrator Thomas Pyke last week told a congressional subcommittee that the Bush administration is committed to keeping Landsats 4 and 5 operating until a policy review of the programme is completed. The new National Space Council, chaired by Vice-President Dan Quayle, is coordinating the effort to find money in the budgets of federal agencies that use Landsat data.

Although still operational, SPOT is beginning to show signs of ageing, and a decision will soon have to be made whether to launch its younger sister, SPOT 2, as planned, later this year. To delay the launch would save money in the short term for the financially stretched French space programme, but the gamble could be too risky.

Seen as a white elephant by all other European countries except Sweden and Belgium, SPOT was financed and built almost entirely with French money. Now, with more than 300,000 high-resolution images in its owners' archives for sale to anyone who wants them for as little as $450, SPOT has convinced even sceptics of its value.

The US Department of Defense came to respect SPOT two years ago, when *Aviation Week and Space Technology* published photographs bought over the counter from SPOT Image Corporation that showed Soviet naval installations. The Pentagon was worried by the realization that anyone could also buy pictures of defence installations in the United States.

SPOT 2 should have been launched last year, but a long setback in the European Ariane rocket launch programme meant that SPOT 1 was kept in service. Although it still sends 700 images each day, one of its two data buffers is out of action and a faulty sensor in each of the two high-resolution cameras has reduced image quality. If the other buffer should fail, details of some of the most strategic par of the globe would be unavailable, and th satellite's commercial value would b drastically reduced.

The French space research cent (CNES) has booked a place for SPOT aboard Ariane flight 34, due in Septen ber. But the French space programme heaving under the burden of recent finar cial commitments to the European Spac Agency and CNES would like to delay th launch so long as SPOT 1 is working wel Although CNES says that SPOT 2 coul always be launched at short notice, if th flight 34 option is not taken up, a failure i SPOT 1 would leave France with only a exotic photograph album to sell unt another launcher is free, which might no be for several months.

Peter Coles & Joseph Palca

* *SPOT: Deux Yeux Braqués sur la Terre*, Bernard Cervelle, Presses du CNRS, Paris, 1989.

ENVIRONMENTAL RELEASE

Pink petunias sow controversy in West Germany

Munich

A CONTROVERSIAL proposal to plant 40,000 genetically engineered pink petunias has been approved by a West German panel, but the future of the experiment remains in doubt. It is the first time that release of a plant containing recombinant DNA has been approved in West Germany. But the federal Health Ministry must pass judgement on the experiment before planting can begin.



Saedler — expressing a lack of optimism.

The experiment itself is not expected to pose a danger to the environment, but opponents fear that it will convince the public that all release experiments are harmless. The experiment is important because it should force better definition for each phase of the now vague licensing procedure. Challenges to approval could come from one of several offices within the federal Health Ministry, the federa Environment Ministry or the *Land* of Nort Rhine-Westphalia.

Biological release is a controversial issue in West Germany, as shown by the storm of protest that greeted Bayreuth geneticist Walter Klingmüller when, in 1987, he carried out an unauthorized experiment with nitrogen-fixing *Rhizobium* bacteria (see *Nature* 328, 568; 1987). In 1985, there was a bomb attack on the Max Planck Institute (MPI) for Breeding Research in Cologne, which has planned the petunia experiment.

Release experiments must now be undertaken in the legal vacuum preceding the long-awaited introduction into parliament of a 'basic law' for regulating genetic engineering. The Central Commission for Biological Safety, the body that unanimously approved the petunia experiment, can request only voluntary compliance with its regulations.

The experiment will demonstrate the behaviour in petunia of so-called 'jumping genes' (transposons). A maize gene has been introduced into the petunia that turns them an unnatural pink colour. If a transposon should jump into the middle of this coloration gene, it is expected to leave parts of the flower white. Among other things, the researchers hope to tell whether transposons move systematically to particular sites, which may have an important bearing on plant evolution.

The jumping event is expected to occur in only one out of every 5,000 to 10,000 plants. For this reason, the experiment must be performed on such a large scale outside a greenhouse.

Heinz Saedler of MPI, the project leader, says that planting must begin by July at the latest if the experiment is to be done this year. But he is "not too optimistic", given the number of federal and *Land* authorities that might hold it up. **Steven Dickman**

POLISH RESEARCH

Solidarity calls for change

London

POLAND's multi-viewpoint 'round table' of experts, convened to seek a solution to the country's long-standing economic and social problems and from which has emerged the concession of open elections to one of the two parliamentary chambers, is also agreed on at least one thing: the urgent need for improvements in science and education.

At the opening of the subgroup on science and education last month, statements by the co-chairmen, Education Minister Jacek Fisiak for the government/party side and Professor Henryk Samsonowicz, former rector of Warsaw University, for the Solidarity/opposition wing, turned out to be virtually interchangeable.

Both said that Polish science and higher education have been damaged by long years of financial neglect, low wages and the lack of social prestige for teachers, and the restriction on the universities' rights to decide their own staffing policy, research and teaching plans.

The difference between the two spokesmen was chiefly one of timing: Solidarity wants immediate change, the official side would prefer to go more slowly. But by the final meeting of the subgroup last Saturday, a timetable had been agreed.

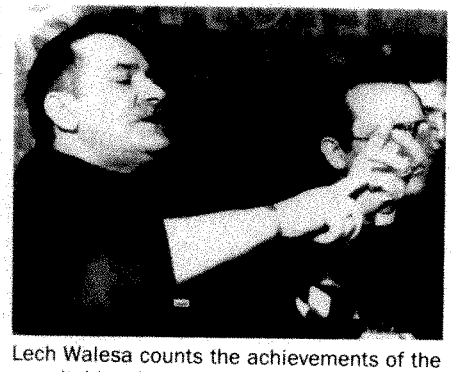
Although the round table includes representatives of all strata of Polish society, from Politburo members to clerics, philosophers, steelworkers and private farmers, its main achievement (and one that took several months to negotiate) has been to bring to the official conference table some of the chief figures from the 'democratic opposition' and the Solidarity movement.

Thus the education and science group includes such scholars as Stefan Amsterdamski and Wladyslaw Kunicki-Goldfinger from the Society of Academic Courses ('Flying University') of the late 1970s, Wiktor Kulerski, Solidarity's main spokesman on education during its legal existence (1980–81) and later a leader of underground Solidarity for the Warsaw region, and Wladyslaw Findeisen, who was removed from his post as rector of Warsaw Technical University in 1985 for refusing to discipline students who took part in pro-Solidarity rallies.

Likewise, the medical subgroup was co-chaired by Dr Zofia Kuratowska, whose haematological clinic was closed in 1985 in reprisal for her work among internees during the martial law period. And Janusz Onyszkiewicz, the Warsaw University lecturer in mathematical logic, resumed his former role of chief press spokesman for Solidarity — at least for the duration of the round table.

But the presence of Solidarity at the round table does not mean that life in Poland is completely back to the *status quo ante* martial law. As Samsonowicz noted, several cases of teachers and lecturers dismissed or penalized on political grounds during the martial law period are still outstanding. Fisiak has accepted a list of these cases "for review" and told a press conference that many other professionals are now being reinstated, there could be no reason why teachers and lecturers should not have the same satisfaction.

This represents a major policy shift. The rationale behind the education ministry's power to intervene against university staffs on political grounds, laid down in the 1985 higher education act, is precisely that teachers and lecturers are not comparable with other kinds of workers because they influence and shape the outlook of the next generation.



Lech Walesa counts the achievements of the roundtable talks last week (AP).

The 1985 act, which largely negated the liberalizations of the 'Solidarity' period and which was introduced in the face of concerted protest from the universities, is now itself scheduled for revision. But shortly after the subgroup began work, Fisiak's promise to restore a measure of autonomy to the universities seemed imperilled when student demonstrations in Krakow took a violent and overtly anti-Soviet note. The reforms, warned government press spokesman Jerzy Urban, depended on university authorities realizing that "autonomy does not mean anarchy".

Solidarity spokesmen have nevertheless been urging that if the banned pro-Solidarity Independent Students' Association (NZS) was reinstated, the students would not have to take to the streets to make their grievances known. The round table, Lech Walesa said, needs three legs in order to stand firm. Two legs had been provided by government promises to legalize Solidarity and the private farmers' organiztion 'Rural Solidarity'. The third 'leg' — legalization of the NZS — was eventually promised, during a meeting last week btween Walesa and Interior Minister General Czeslaw Kiszczak.

Vera Rich

DEFENCE RESEARCH

Industry co-sponsorship

Paris

YVES Sillard, the former French coordinator of the European EUREKA programme seeking industrial co-sponsorship for competitive product research, thinks the principle could be extended to defence research. Sillard, who recently took over as head of the French Committee on Armaments (DGA), would like to see industry put up venture capital for pre-production studies of new military applications, in the expectation that military approval would be rewarded with government production contracts.

Military research now accounts for more than a third of French government research and development spending (about $4,000 million). Sillard believes that as European trade barriers fall in 1992, there will be opportunities for transnational collaboration, even if some domains (such as nuclear arms) would not be suitable. Sillard's idea is being studied by the DGA before being put to other European defence agencies.

Peter Coles

INFORMATION TECHNOLOGY

No action on research

London

THE British government last week rejected calls from the House of Commons Trade and Industry Committee for an increase in the number of undergraduate and graduate courses in information technology (IT) in order to tackle an emerging skills shortage.

The committee had said, in a report on IT, that there are 30,000 unfilled vacancies for people with IT skills and that the shortage was worsening. But the government now says, in a policy paper setting out policy on IT, that measures to combat the shortage have already been introduced. A £43-million programme launched four years ago will by 1990 have provided 5,000 extra places on undergraduate and post-graduate courses, it said.

It also rejected the committee's call for increased spending on research and development in IT, saying that £100 million will be spent by the government next year for this purpose. It stressed that the chief responsibility for financing industrially relevant research lies with industry, and rejected the committee's suggestion that the government should encourage industrial investment in IT by identifying a target level of research. It said that the government would not require that investment be disclosed in company accounts, as this would be burdensome to industry.

The committee had called for reinstatement of the post of minister for information technology, on the grounds that since the post was abolished there has been no significant increase in interest in the subject among ministers. But the government says it is neither feasible nor desirable to locate responsibility for all aspects of IT policy in a single department.

Christine McGourty

Earthquake risk

SIR—The recent earthquake in Armenia has once again focused attention on the need to adhere to some minimal building code in order to minimize earthquake hazards, especially in developing countries in seismically active zones (*Nature* **336,** 625; 1988). This is the only practicable solution for such countries, where the development of facilities and capabilities for data-acquisition and data-processing to monitor the build-up of strain and to predict its release in earthquakes is still far in the future.

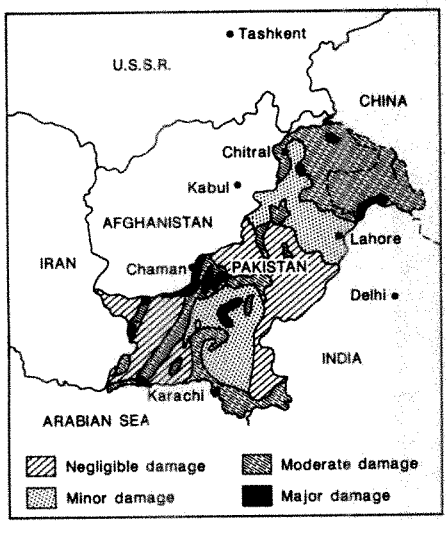
Towards the middle of the next century, the developing countries in Asia with a history of destructive earthquakes will experience a growth of development in communication, energy, irrigation and industry, financed largely by international agencies and executed under the supervision of experts in the countries concerned.

These agencies and experts must make themselves responsible for minimizing natural hazards, of which earthquakes are the most sudden and destructive. They should perhaps specify that a seismic zoning map should be available when a large project is planned.

A seismic zoning map of Pakistan (see below) is being published for the information of international funding agencies and for those involved in structural designs for dams, power and chemical plants and so on in Pakistan.

ABUL FARAH

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Pakistan



This map, constructed from macro-earthquakes data from 1905 to 1979, can be used for risk assessment if one assumes that if ground motion of a certain intensity is experienced once in a certain area, it is likely to be experienced again in that area. Map adapted from Building Code of Pakistan, Ministry of Housing & Works, Islamabad, 1986, pp 11–38 to 52.

Looking-glass land

SIR—Our solution of the molecular structure of foot and mouth disease virus (*Nature* **337**, 709; 1989) involved an intriguing number of coincidental connections with the life and career of Lewis Carroll, the mathematician and author of the Alice in Wonderland books. He was born and spent his early years in Daresbury, the location of the SERC synchrotron source where the X-ray diffraction data were collected. He later moved to Oxford to teach mathematics and it is there that the data were analysed and the structure of the virus solved. Carroll also owned a house in Guildford, which is close to Pirbright, where the virus was grown and crystallized. The final link was provided by the representation of the virus structure featured on the cover of *Nature*. This picture is as the virus would have been seen by Alice through the looking-glass — it was printed as the mirror image of the true structural organization of the particle.

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Aluminium limits

SIR—It has been known since 1983 that a blood serum level of aluminium in excess of 0.1 mg per l presents a significant risk of neurological damage[1], yet the permissible level of aluminium in European Communities domestic water remains at 0.2 mg per l. Are we not expecting to much from the European kidney?

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1. Savory, J. & Berlin, A. *Annals of clinic. Lab. Sci.* 444–451 (1983).

Improbability

SIR—Respondents to Bruce Denness's query (*Nature* **336,** 614; 1988 and **337,** 498; 1989) are surely correct that scientists need not be too much concerned with far-fetched theories that are fabulously contrived to explain phenomena. But the fantastical context of the discussion should not be permitted to obscure a serious, but related, scientific problem.

One reason to reject the spontaneous or wilful creation of the Universe in the recent past is that we have a good explanation that hinges improbabilities of the recent past on the even more improbable (in the sense of lower entropy) less-recent past. But in a Big-Bang cosmology, this particular buck can be passed back only so far. How did the earliest times come to be so improbable? Roger Penrose has estimated[1] that the original conditions of our Universe, if generated randomly, had a likelihood comparable to 1 part in 10 to the power of 10^{123}. It is not clear that such a staggeringly improbable initial condition is any easier to arrange than admittedly preposterous, direct fabrication of a recent state of the Universe replete with falsified history.

WILLIAM ECKHARDT

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1. Penrose, R. "Time-Asymmetry and Quantum Gravity" in *Quantum Gravity 2.* Isham, Penrose and Sciama) (Oxford, 1981).

Anthropic principle

SIR—I was surprised to learn in the slightly misleading report from M. Abramowicz and G. F. R. Ellis (*Nature* **337**, 411–412; 1989) that a feature of the Venice Conference on cosmology and philosophy (18–19 November 1988) had been my abandonment of the final anthropic principle. In fact, my talk was concerned primarily with other subjects. However, the documented discussion following other speakers reveals that the scientific basis of this proposal was presented and defended. It is particularly interesting in view of recent ideas about information processing and complexity. For a truer picture, I refer the interested reader to the conference proceedings which will be published by Cambridge University Press under the editorship of U. Curi.

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Retraction test

SIR—I agree with John Maddox (*Nature* **338,** 13; 1989) on the importance of full retraction by an author who discovers nontrivial mistakes in his or her previously published work. It is a litmus test of scientific integrity.

To any researcher hesitant about publishing a retraction when necessary, I would say that I can immediately think of colleagues whose reputations went up in my estimation when they published retractions.

I was horrified to read that some funding bodies apply the opposite criterion. This must be stopped.

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Risks from ionizing radiation

R.H. Clarke and T.R.E. Southwood

For the public in the United Kingdom — and the United States — natural radon is a far greater radiation hazard than are emissions from nuclear installations.

RADON indoors now accounts for nearly half of the average UK population exposure to ionizing radiation. We believe that the extent of the variation in exposure to radon in the United Kingdom and elsewhere and its magnitude in relation to exposures from other natural and artificial sources needs to be more widely recognized. Such information should influence attitudes to risk and the allocation of resources for programmes of radiological protection. We cannot help but observe the large investment being made in the United Kingdom to reduce doses from the Sellafield nuclear reprocessing plant[1], in comparison with that being made to reduce exposure from indoor exposure to radon[2].

The National Radiological Protection Board monitors the exposure of the UK population to all sources of ionizing radiation. We have just completed our latest assessment[3], which is based as far as possible on measurements of exposure from natural background radiation, medical diagnostic procedures, fallout of radionuclides and occupations involving use of radiation. Some exposures are too low to be measured, in which case we calculate doses using environmental and dosimetric models, such as consumer products and the average dose from effluents from nuclear power stations.

We estimate that the average exposure within the United Kingdom is now 2.5 millisieverts (mSv) per year compared with just under 2.2 mSv per year when we last reported[4]. The increase is principally due to a re-assessment of exposure to indoor radon gas as a result of our national survey[5] of the levels and the re-evaluation of the dose–intake relationship for radon and its daughters. The contributions of various sources of radiation in 1988 are listed in Table 1 in descending order of importance, natural sources being identified and the doses from 1984 being given for comparison. There have been reductions in occupational exposure and from nuclear effluents in the period since our 1984 report, but these are small compared with the increased estimate for radon.

In the United States, the national average exposure to ionizing radiation has been estimated recently to be 3.6 mSv, more than 1.4 times the UK average[6]. (The breakdown of components of dose in the United States is also shown in Table 1.) Medical exposures to individuals are higher in the United States than in the United Kingdom, as are doses from consumer products and other miscellaneous sources. It is clear that higher average radon exposures in the United States account for most of the difference from the UK figures, although the US distribution is not yet well known.

National averages are useful for broad comparisons, but any average conceals a wide range. The dose received by single individuals may depart markedly from the average, depending on their mode of life and geographical location. Therefore, in the figure, we contrast doses received by different groups in the UK population with the national average figures. The area of each pie diagram is proportional to the total dose received, and the slices represent the percentages from the various sources.

TABLE 1 Contributions to average radiation exposure (mSv)

UK 1988	UK 1984	Source	USA 1987
1.2	0.7	*Radon from earth materials (98 per cent from indoor exposure)	2.0 †
0.35	0.40	*Terrestrial γ-rays	0.28
0.30	0.37	*Ingested natural radionuclides	0.39
0.30	0.25	Medical exposures	0.53
0.25	0.30	*Cosmic rays	0.27
0.10	0.10	*Thoron from earth materials	—
0.01	0.01	Fallout (including Chernobyl)	0.0006
0.01	0.01	Miscellaneous sources	0.05-0.13
0.005	0.009	Occupational exposure	0.009
0.001	0.002	Radioactive effluent discharges	0.0005
TOTAL 2.5	2.2		TOTAL 3.6

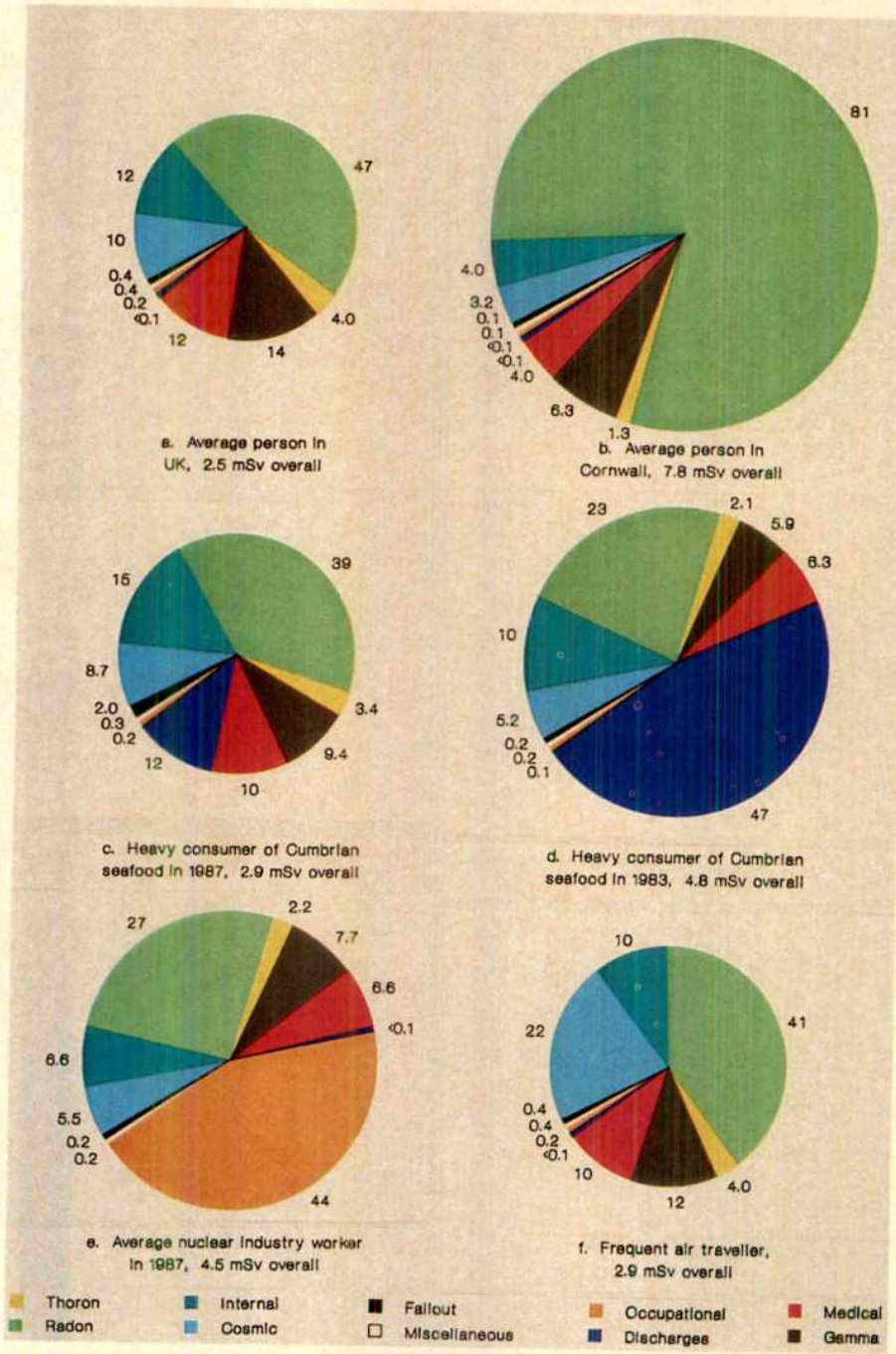
* Natural sources
† Includes thoron

In general, the highest doses in the United Kingdom arise in Cornwall and Devon in the south-west of England. By comparison with the UK average (*a* in the figure), the average dose received in Cornwall (*b*) is slightly more than three times higher (7.8 compared with 2.5 mSv), principally because of the nature of the local geology. The terrestrial γ-radiation is slightly higher, but the main contribution to the increase arises from the effects of the inhalation indoors of radon and its daughters (81 per cent of the total). Radon in houses arises primarily through seepage from the underlying ground, caused by the slight under-pressure indoors. Better insulation reduces ventilation in houses and thus increases radon exposure because of the lower rate of changes of fresh air[7]. But apparently obvious remedies, such as the use of an extractor fan, may cause an increase in under-pressure and draw more radon from the ground, making the situation worse[2].

We know of parts of Cornwall, and some other areas of the United Kingdom, where the exposure of individuals can rise to more than 20 mSv in a year when more than 90 per cent of the dose results from indoor radon[7].

The public and the media in Britain have generally been more concerned with the safety of artificial sources of radionuclides, in particular the effects of discharges to the Irish Sea from the Sellafield reprocessing plant in Cumbria in the north of England. Those receiving the highest doses in this region are the avid consumers of seafoods collected off the Cumbrian coast near the plant (*c* in the figure), whose dose is increased to 0.33 mSv compared with the UK average of 0.001 mSv[5]. Nevertheless, the total exposure of these people is 2.9 mSv per year, not much higher than the national average, and much less than the value of 4.8 mSv per year (*d* in the figure) which we reported in 1984 (ref. 4). These lower doses result from the reduced discharges of radionuclides from the nuclear reprocessing plant at Sellafield achieved since 1984.

We also note the increase in natural internal exposure associated with high shellfish consumption (*c* in the figure) from the increased intake of natural α-emitting radionuclides (mainly polonium-210) that are concentrated by shellfish. This increase, which is unrelated to any man-made source of radionuclides, can raise the annual internal dose of an individual by 0.14 mSv (from 0.30 to 0.44 mSv), just under half the additional dose now received by those most exposed to the Sellafield discharges. Because of the pattern of rainfall in May 1986, people living in Cumbria (and in other parts of western Britain) are still exposed to slightly higher than average doses of radiation because of residual fallout from the



Variations of annual radiation dose in the United Kingdom compared with the average. Pie areas are proportional to dose and percentage segments to sources.

reactor accident at Chernobyl (0.05 mSv in 1987 compared with the average of 0.01 mSv).

The effect of occupational exposure on the pattern of total dose is shown in *e* in the figure, which is the dose for a typical UK nuclear industry worker in 1987. Occupational exposure does, of course, vary from one individual to another, as well as for the same individual at different times of life. (The average dose of 2 mSv is obtained from about 20,000 reactor workers receiving doses averaging less than 1 mSv per year together with more than 5,000 reprocessing-plant workers averaging about 5 mSv per year[3].)

Other features of modern life have a significant effect on dose received — because of the reduction in the screening effect of the atmosphere with height, for example, air travel increases exposure to cosmic radiation[9]. Frequent air travellers who, in a year, fly for 100 hours (about five subsonic return flights between Britain and the west coast of North America, or a weekly flight between London and Scotland or a nearby European country) would increase their dose from that source from 0.25 to 0.65 mSv per year. This would lead to a total dose of 2.9 mSv per year as shown in *f* in the figure — the same dose as received by those most exposed to discharges from Sellafield.

Our new survey[3] shows that radon is th greatest single source of radiation to th population. Radon and its daughters ar α-emitters, which are more biologicall damaging than β- and γ-radiations. A there a real risk of lung cancer from rado exposure in the home? There are dat from studies on uranium miners who hav been chronically exposed to radon and it daughters[9], and it is encouraging that ou estimate for the risks of lung exposur derived from the Japanese atom-bom survivors[10] is close to the figure for th uranium miners. Experiments on rodent exposed to radon result in similar risks and confirm a linear relationship betwee risk and dose[11].

Depending on the exact risk factor applied, radon exposure in the Unitec Kingdom might be responsible for 6 pe cent or more of the annual incidence o lung cancer (41,000). We recommend ar action level for radon exposure in home corresponding to 20 mSv in a year[12], anc similar action levels are being considered in Sweden and the EEC[13]. In the Unitec States, action is recommended at 7.5 mSv in a year[14], and there is legislative pressure for abatement as well as for programmes of intervention and research[15]. Our advice in 1987[12] was that lifetime exposure to radon at its action level would give rise to about a 2 per cent chance of an individual dying of lung cancer. It is now clear that this figure has to be revised substantially to at least 5 per cent, and that action to reduce the risks from radon are even more urgently required. □

R.H. Clarke is Director and Sir Richard Southwood Chairman of the National Radiological Protection Board, Chilton, Didcot, Oxford OX11 ORQ, UK.

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Space Telescope ready at last

The long delay in launching the Hubble Space Telescope, caused by the Challenger disaster, has given the Space Telescope Science Institute time to develop strategy, and to win the respect of the community.

Baltimore

WHEN the Hubble Space Telescope (HST) is finally launched, maybe in December, the most sophisticated automatic telescope yet built will be in a 90-minute orbit 300 miles above the Earth, but its users will be left firmly on the ground. The Space Telescope Science Institute (STScI) here will be the nearest they can get to it, which may be regarded as a way of carrying to its extreme the growing belief among those who operate major telescopes that the presence of live astronomers is often an inconvenience, sometimes even a hazard.

As at observatories everywhere, users will submit proposals which will be assessed for scientific merit and their particular suitability for the instrument they intend to use. Successful proposals will be worked up in detail, with the help of staff astronomers at the STScI, and interleaved with all the other successful proposals to make the most efficient use of available observing time and instrumentation.

But HST has to fulfil expectations beyond the relatively straightforward hopes of scientists: it (or more particularly the people who operate it) must prove that the money and anxiety spent on it, especially during the enforced hiatus caused by the Challenger disaster, have not been in vain. The US Congress, and US taxpayers generally, will be looking eagerly over the users' shoulders for surprises and novelties. Some astronomers may regard this element of showmanship as an intrusion; fortunately, the director of STScI, Riccardo Giacconi, is not one of them. But what spectacles to provide?

The charter of STScI contains the useful provision that its purpose is to "conduct" an observing programme on the Space Telescope. This gives the director the freedom to act either as the passive arbitrator of proposals or as the dictator of what observations should be made. The more pharaonic extreme will be valuable in the early days.

Even if the Space Telescope is launched as intended during December this year (and difficulties with the imminent launch of Discovery cast some doubt on this), it will not be ready to spring instantly to life. The first six months have been allocated to commissioning the telescope and its various instruments. This period can be stretched by one more month, but after that STScI intends to gain control of the Space Telescope because, as one astronomer said, the engineers would carry on commissioning the instrument for the whole of its fifteen-year lifetime, given the chance.

During commissioning, trial observations will be made with the telescope's six instruments (two cameras, two spectrographs, a photometer and a fine-guidance system for astrometry). Some data and images will therefore be coming down to Earth before observations officially begin. Will these do for Congress?

STScI is working under a written obligation from NASA that any such 'first light' images used for promotional or other purposes must specifically not have any scientific interest. The supposed danger is that if, for example, *Nature* were to publish on its cover sometime next January an unprecedentedly fine picture of a nearby globular cluster, in which the central stars could be made out individually instead of blurring together, then eager astronomers around the world would get out their plastic rulers and start counting stars, uncovering long-held secrets about the internal dynamics of dense stellar systems and depriving whichever group of astronomers had the first proposal to do the same thing of their chance for glory. STScI is thus faced with the task of producing first images which are both spectacular and uninteresting, a riddle not yet solved.

Once the observing programme begins in earnest, scheduling tactics will be more conventional, but some special difficulties remain. For the Space Telescope, the cycle of day and night is replaced by the 90-minute orbital period, and although an object near the North or South pole can be observed continuously, those parts of the sky are generally empty of interesting objects: the galactic plane is close to the plane of the Solar System and the Earth's Equator, which means that most observing will have to be done in the 45-minute portions of time when the Earth is not in the way. On top of that, the Space Telescope must not be allowed to point at the Moon or the Sun, which would be fatal to some of the instrumentation.

The speed of the orbit combined with the telescope's lack of speed in slewing across the sky (its direction can be changed only a little faster than the minute hand moves on a clock) combine to make efficient scheduling difficult. During the launch delay, STScI has developed scheduling algorithms to maximize the time that observations are being made.

Switching from one instrument to another is a headache, involving as it does choosing between six detectors, selecting one of about fifty filters, changing filters and detectors during an observing run, and perhaps running two instruments simultaneously. STScI will be pleased to be making observations one-third of the time.

Deciding what observations to make is the final part of STScI's strategic puzzle. Ultimately, one of the Space Telescope's greatest virtues may lie in its ability to perform long-term projects, such as dissecting and cataloguing distant clusters of galaxies or searching for infrared emission from nearby subdwarf stars.

The deadline for the first round of proposals passed last October, and almost 600 proposals from 1,500 astronomers in 30 countries have been received. About 15 per cent of the observing time is guaranteed to investigators from the European Space Agency, which has provided the Faint Object Camera and the solar panels for HST, but the 15 per cent target is intended only as a long-term average, and there will be no lawsuits if the proportion during the first year or so is a little amiss.

Observing time is oversubscribed by about seven to one for this first period, which has allowed Giacconi and his staff to exercise their discretionary powers to slant observations in chosen directions without sacrificing quality. Many of the programmes that STScI favours are of cosmological importance: finding Cepheid variable stars at the distance of the Virgo cluster of galaxies, for example, would bypass several rungs of the cosmological distance ladder and could at a stroke reduce our ignorance of the Hubble constant and the age of the Universe.

Giacconi has understood from the outset that the ability of STScI to impose, to some extent, its designs on the rest of the astronomical community depends on the standing of his institute. STScI scientists win about 20 per cent of the observing time at Kitt Peak National Observatory.

It is a trite comment now that the years of delay to HST's launch have been beneficial, allowing the instruments to be improved, a satisfactory number of guide stars to be catalogued, and scheduling problems to be overcome. But more important is that the delay has allowed STScI to become an astronomical institution of the first rank whereas, had HST been launched on time, it might have been merely a service station. **David Lindley**

HUMAN IMMUNODEFICIENCY VIRUS

Revving up gene expression

Michael R. Green and Maria L. Zapp

THE expression of eukaryotic protein-coding genes can be regulated at a variety of levels from transcription to translation. It has been largely assumed, however, that the transport of the fully processed messenger RNA from the nucleus to the cytoplasm (mRNA nuclear export) is a constitutive step. Studies of the human immunodeficiency virus (HIV) reported by Malim *et al.* on page 254 of this issue[1] suggest that this assumption is no longer tenable, and may stimulate the quest for cellular proteins that regulate the export of RNAs from the nucleus.

All replication-competent retroviruses, including HIV and the somewhat similar human T-cell leukaemia virus (HTLV-I), have three essential structural proteins: the group-specific antigen, the envelope protein, and the reverse transcriptase, encoded respectively by the *gag*, *env* and *pol* genes. HIV and HTLV-I are, however, souped-up retroviruses, which also encode unique proteins that regulate viral gene expression. Two HIV regulatory proteins are encoded by the *tat* and *rev* genes, and the corresponding HTLV-I proteins by the *tax* and *rex* genes[2] (see Fig. 1).

The HIV Tat and HTLV-I Tax proteins act at the transcriptional level to increase synthesis of both the structural and regulatory viral proteins. The HIV Rev and HTLV-I Rex proteins, however, selectively increase the synthesis of the structural proteins later in infection, thereby maximizing the production of viral particles. The basis for this selective action lies in a second unique feature of HIV and HTLV-I: both viral genomes contain at *least* two introns. Both introns are present in the *gag–pol* mRNA, whereas one intron is removed to produce the *env* mRNA, and both are spliced out of the mRNA(s) encoding the regulatory proteins (Fig. 1).

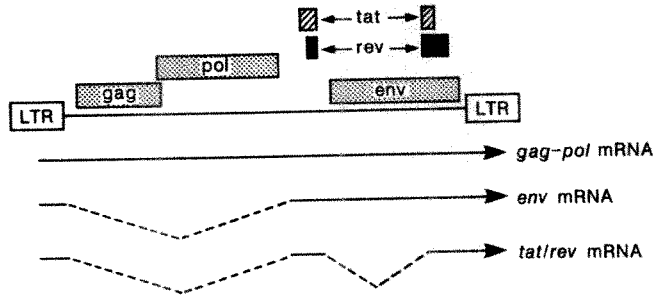


FIG. 1 A simplified version of HIV gene expression. The regulatory proteins of HIV (and HTLV-I) are expressed from largely overlapping reading frames. The HIV proviral genome is shown bracketed by the two LTRs. The viral protein-coding regions are shown above. A schematic representation of the splicing patterns of the viral mRNAs is shown below. Exons, solid lines; introns, dashed lines.

Rev and Rex augment the production of viral structural polypeptides by increasing the cytoplasmic concentration of the intron-containing *env* and *gag–pol* mRNAs. One way Rev and Rex could effect this increase is by specifically inhibiting splicing. This would raise the amount of unspliced *gag–pol* mRNA and singly spliced *env* mRNA at the expense of the *tat/rev* mRNA, whose production requires two splicing events. Alternatively, Rev and Rex could act by specifically accelerating the nuclear export of the *env* and *gag–pol* mRNAs. (These two possibilities are not mutually exclusive as accelerated nuclear export is itself a mechanism for inhibiting splicing, a point we shall return to.)

Malim *et al.*[1] present three lines of evidence, which together argue that Rev does not directly inhibit splicing. The first set of experiments asks what is the RNA sequence(s) required for the effect of Rev (Rev-responsive region; RRE)? The existence of an RRE is implied by the fact that the viral *env* mRNA, but not a cellular mRNA presursor (pre-mRNA) such as insulin, is responsive to Rev. To test whether the RRE corresponds to a viral splicing signal, chimaeric genes were constructed in which each of the known splicing signals of the viral *env* RNA was replaced by the equivalent insulin pre-mRNA signal. All of the chimaeric pre-mRNAs were still responsive to Rev, indicating that no viral splicing signal is uniquely required for Rev action. Next, Malim *et al.* mapped the RRE to a portion of the *env* coding sequence that is spliced out of the *tat/rev* mRNA (intron 2), and showed that it functions following translocation to several positions within intron 2, and even when moved to the exon. The ability of the RRE to work independently of its position within the RNA, and particularly within the exon, makes it unlikely that Rev acts at the level of splicing.

In a second set of experiments, Malim *et al.* measured the ratio of unspliced-to-spliced *env* RNA in nuclei and cytoplasm. If Rev directly inhibits splicing, the increased ratio of unspliced-to-spliced *env* RNA in the cytoplasm should be paralleled in the nucleus. However, Rev has little effect on the ratio of unspliced-to-spliced *env* RNA in the nucleus, arguing that Rev does not directly affect either splicing or nuclear stability. (Note, however, that this conclusion relies upon assumptions regarding the rate-limiting step in splicing, and cytoplasmic mRNA production, and potential inter-relationships between splicing and export; see below.) Finally, Malim *et al.* tested the splicing inhibition model by asking whether Rev can act on a pre-mRNA rendered unsplicable by removal of the 5′ splice site. They find that Rev increases the cytoplasmic concentration of this unsplicable RNA, a result more compatible with the nuclear export model than the splicing inhibition model.

Comparable experiments have not yet been performed with the HTLV-I Rex protein, but it is probable that Rev and Rex act similarly. The HTLV-I Rex protein can functionally substitute for the HIV Rev protein[3], an intriguing observation in the light of the fact that these two proteins lack amino-acid similarity.

Although Rev may not directly inhibit splicing, by accelerating nuclear export Rev inhibits splicing indirectly: export separates the pre-mRNA from the nuclear-localized splicing factors. Splicing occurs in the nucleus following assembly of the pre-mRNA into a large complex, the spliceosome, which contains multiple small nuclear ribonucleoproteins (snRNPs). The spliceosome may also serve to retain RNAs in the nucleus by, for example, preventing the RNA from productively interacting with the nuclear pore, the normal avenue of egress[4,5]. In this sense, spliceosome assembly and nuclear export are competing processes (Fig. 2), a notion supported by recent experiments in yeast in which mutations that prevented spliceosome assembly facilitated nuclear export[6].

Upon splicing, the mRNA is released from the spliceosome, which remains primarily associated with the intron. The mRNA, but not the excised intron, is then free to be exported. This 'spliceosome-retention' model can explain the selectivity of RNA export: for a typical cellular pre-mRNA, the spliced mRNA product is exported, whereas the unspliced pre-mRNA and the other product of the splicing reaction, the excised intron, are retained in the nucleus.

Retroviral pre-mRNAs are spliced notoriously inefficiently, perhaps because of specific inhibitory *cis*-acting elements[7,8]. This inefficiency is probably critical to the

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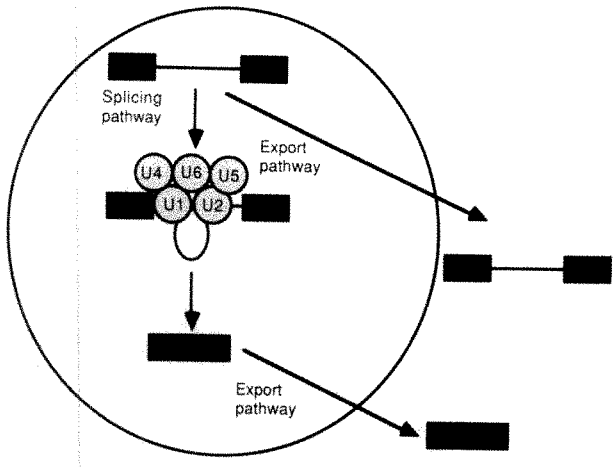


FIG. 2 Splicing and export pathways in the eukaryotic cell nucleus, showing potential processing pathways for a pre-mRNA in the nucleus. The pre-mRNA is shown with boxes representing exons, and lines representing the intron. In a splicing pathway, the pre-mRNA is assembled into a spliceosome. In the spliceosomal complex, U1, U2, U4, U5 and U6 snRNPs are shown as circles. Some of the snRNP–snRNP contacts in the spliceosomal complex are arbitrarily drawn.

propagation of the virus, because incompletely spliced and unspliced RNAs are required to encode structural proteins and to serve as genomic RNA. Conceivably, Rev may be able to work only upon inefficiently spliced pre-mRNAs. For example, Rev may act before spliceosome assembly, and efficiently spliced pre-mRNAs are probably assembled rapidly into spliceosomes. In fact, electron microscopy has shown that the spliceosome assembles immediately on the nascent transcripts of some cellular genes[9].

A more detailed understanding of the mechanism of Rev (and presumably Rex) may be hampered by our limited knowledge of mRNA nuclear export. For example, we do not even know whether mRNAs require a specific export signal, or whether, if not encumbered by a spliceosome, they are exported by default. The existence of single-base substitutions that can prevent export of transfer RNAs[10-12], argues that at least some RNAs harbour specific export signals.

How might Rev accelerate the rate of export? Recent immunofluorescence experiments may provide a clue. Surprisingly, the HTLV-I Rex protein is localized in the nucleolus rather than the nucleoplasm or nuclear envelope[13], perhaps implicating the nucleolus or a nucleolar constituent in mRNA nuclear export.

Although Rev might just be a quirk of HIV, studies of eukaryotic viruses have often provided the first glimpses into cellular regulatory mechanisms. If history repeats itself, Rev and Rex are the harbingers of cellular regulatory proteins waiting to be discovered. □

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MATERIALS SCIENCE

Fractal dimension and fracture

Robert W. Cahn

How rough is a fracture surface? This question is in the domain of the craft of fractography, which seeks to elucidate the mechanisms of fracture by studying the topography of a broken object. Somewhat akin to phrenology, some sceptics might say. Nevertheless, in two new studies, Mecholsky, with Mackin[1] and with Passoja and Feinberg-Ringel[2], shows that it may be possible to scale the fracture toughness of fairly brittle materials by just such an exercise in fractographic analysis, exploiting the concept of fractals.

Fractals were invented — or at any rate clearly formalized — by Mandelbrot[3,4]. The term refers to any boundary or surface which remains self-similar as the scale of examination is magnified. Figure 1 gives an example of such a boundary. For this shape, a 'Koch island', drawn to be always of constant area, the length of the perimeter is increased by a factor of 1.5 from one drawing to the next, if the length of the ruler unit used to measure it is decreased by half, and the image resolution grows by a factor of four. If the logarithm of the total perimeter length is plotted against the logarithm of the length of the ruler unit (the resolution), a straight line or 'Richardson plot' is obtained, the slope of which was treated by Mandelbrot as a fractional, fractal, dimension: for a boundary as in Fig. 1, this dimension is between 1 and 2 — in this case, 1.5.

Similarly, a rough surface which is self-similar has a dimension between 2 and 3. To obtain this fractal dimension, it is usual either to make serial sections perpendicular to the surface ('vertical' sections) and examine the shape of the section boundary, or to section parallel to the surface and examine the perimeters of the 'islands' and 'lakes' which are in effect contours of such 'horizontal' sections.

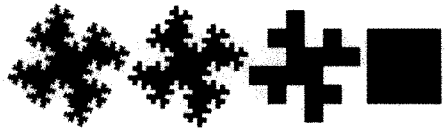


FIG. 1 A Koch island with a perimeter of fractal dimension 1.5 (from ref. 5).

The original concept of fractals was restricted to self-similar outlines only, but more recently the concept has been extended to more ordinary boundaries and surfaces of varying roughness; thus, Fig. 2 shows four outlines generated by Russ[5] by a rather involved computer algorithm, with four different fractal dimensions. In such cases, the corresponding Richardson plot has a straight central part with curved regions at either end. (Such shapes should really be called 'pseudo-fractals', but that term does not appear to have been used.) Stereologists still disagree about the appositeness of the horizontal section approach[6], but in a recent study of fracture surfaces of ceramics, Mecholsky and Passoja[7] found that the horizontal and vertical methods gave closely similar fractal dimensions.

The studies of Mecholsky and colleagues[1,2,7] on the relation between fractals and fracture were stimulated by two apparently independent initiatives. One was a fractographic study by Mandelbrot *et al.*[8] of broken specimens of maraging steel. The impact toughness of 300-grade maraging steel in various states of heat-treatment increases steadily as the fractal dimension of the fracture surface decreases towards 2 (it becomes steadily smoother)[8]. It is apposite that Mandelbrot should have been involved in this early study because, as he says, he coined the term fractal "in explicit cognizance of the fact that the irregularities found in fractal sets are often strikingly reminiscent of fracture surfaces in metals (though not, for example, in glass)". Although he does not say so, one should not lose sight of the fact that the word fraction has the same Latin root as fracture, and fractals have fractional dimensions.

The second study was by Beauchamp and Purdy[9], who examined the changes in fracture toughness and fracture morphology of chert (flint) as it is heat-treated at progressively higher temperatures. It turns out that chert heated to 500 °C is considerably less tough than untreated material; the heated chert also has a much smoother surface. (This implies that heated chert is easier to chip and shape into tools, and indeed there is archaeological evidence that chert was commonly heated in prehistoric times.) Heating coarsens the exceedingly fine grain of the chert and also apparently enhances the strength of the intergranular bond: unheated chert fractures along the boundaries of the fine grains, heated chert breaks transgranularly. But the unheated material is nevertheless tougher because fracture is constrained to follow a very tortuous path.

Beauchamp and Purdy did not explicitly invoke the fractal concept in their study,

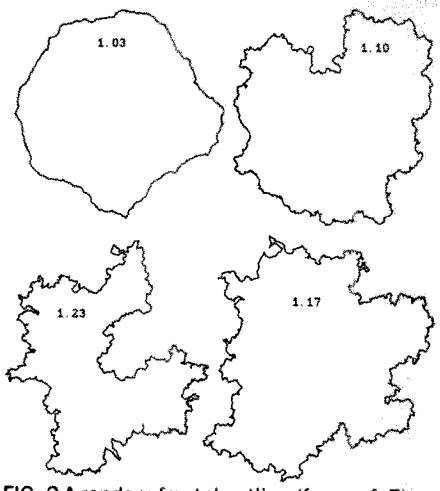


FIG. 2 A random fractal outline (from ref. 5).

but in the new work[1,2] by Mecholsky and co-workers, their original samples are subjected to a proper fractal analysis, using both horizonal and vertical methods of analysis. It is quite clear that the fracture toughness, K_c, of chert increases as the fractal dimension increases — as the fracture surface becomes rougher. This is the exact converse of what was found for maraging steel. (The relationship for chert is in the form of a linear log–log plot of K_c against fractal dimension.)

The authors also indicate, by means of a figure relating the fractal dimension of the fracture surface to fracture toughness for several ceramics (oxides, silicates, chalcogenides) that this form of relationship is universal for such highly brittle materials, though the results bunch into several distinct material groups rather than falling along a single master line. This research is itself a continuation of earlier work[7].

We thus have the intriguing fact that the relationship between fracture roughness (fractal dimension) and fracture toughness is diametrically opposite for ductile fracture (maraging steel) and for brittle fracture (chert and polycrystalline ceramics). For chert, where fracture is governed by the spreading of cracks, the toughening influence of factors making for rough fractures has been qualitatively explained. For ductile fracture, which can take place by the progressive creation, growth and merging of minute voids, the relationship is clearly different. Mandelbrot *et al.*[8] seek to interpret their findings in terms of percolation concepts applied to the filaments remaining intact between voids, but it must be said that their argument is not easy to follow. It is to be hoped that in future work, Mecholsky and colleagues will address the piquant distinctions between ductile and brittle fracture which these studies have uncovered. □

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MICROBIOLOGY

Bacteria that thrive in toluene

Howard Goldfine

TOLUENE is widely used in the laboratory to permeabilize and kill bacteria. Thus, the isolation of a bacterial strain described by Inoue and Horikoshi on page 264 of this issue[1], which not only resists the toxic actions of toluene, but grows luxuriantly in the presence of emulsions containing up to 50 per cent of this solvent, represents a significant achievement. In a rich medium, Inoue and Horikoshi find that growth in the presence of toluene is about two-thirds as rapid as that obtained in its absence. It should, however, immediately be noted that the newly isolated strain only tolerates toluene rather than using it as an energy source.

The organism, identified as a strain of the gram-negative aerobe *Pseudomonas putida*, an organism known for its protean degradative capabilities, was isolated after screening 750 soil samples collected in Japan. The only hint concerning the site of its isolation is that the soil was obtained from Kyushu, Japan's large southern island. It is not clear whether these soils are contaminated with organic solvents.

The isolation of organisms resistant to aromatic hydrocarbons and other industrial products has implications for biodegradation and biotransformation. The annual global production of benzene and toluene each exceeded 10^9 gallons in 1980, and these materials are likely to be found in soils and sediments[2]. The availability of organisms that can withstand the toxic effects of these organic solvents and participate in their degradation or transformation is clearly desirable. *P. putida* contains chromosomal genes encoding the enzymes involved in the degradation of aromatic compounds by the so-called ortho cleavage pathway, and strains have been isolated that contain the common TOL plasmids encoding the genes for the enzymes of the catechol meta cleavage pathway[3]. It would seem to be just a short step to bring together these degradative resistance characteristics. The isolated strain is also resistant to styrene, *p*-xylene, ethylbenzene, cyclohexane, *o*-dichlorobenzene, propylbenzene and various alkanes up to isooctane, thus there is wide scope for producing both solvent-tolerant and solvent-degrading strains. The marriage of these two desirable traits may be less than harmonious, however. The alterations in the cells of the toluene tolerant strain that make growth in solvents possible could shield the cell from these solvents so perfectly that little penetration to the locations of degradative enzymes would be possible.

The authors of the paper in this issue, one of whom is a member of the Superbugs Project of the Research Development Corporation of Japan, have begun a genetic analysis of the solvent resistance of *P. putida*. They have isolated a series of mutants of their new strain that are not resistant to toluene, but retain resistance to solvents of lower polarity in the order toluene > *p*-xylene > cyclohexane > hexane.

By examining the partitioning index of these and other substances in a standard octanol:water mixture, they can predict accurately the resistance of these mutant strains to other compounds in the polarity scale. In addition to mutant strains of *P. putida*, they can order other microorganisms in a similar hierarchy of solvent-tolerance, in which growth in a solvent of a given polarity indicates tolerance to solvents of lower polarity. Genetic analysis combined with biochemical and morphological studies should provide useful insights into the nature of solvent tolerance in these bacteria.

The outer membrane of gram-negative bacteria is important in determining the penetration of hydrophobic molecules through cell membranes, and consequently in determining the resistance of these bacteria to dyes, detergents, bile salts and hydrophobic antibiotics. Mutations in the structure of the lipopolysaccharide of the outer membrane can profoundly affect permeability of these molecules, but these effects are complicated by concomitant changes in the amounts of specialized pore-forming, outer-membrane proteins[4]. Studies of the outer membranes of these solvent-resistant strains of *P. putida* may be rewarding.

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CELL MOTILITY

Networks from mutants

Dennis Bray and Juri Vasiliev

THOSE of us who earn a living from the cytoskeleton feel a little threatened. We had always asserted (rather too dogmatically, as it now seems) that actin and actin-binding proteins were the motor by which animal cells crawl from one location to another. And yet André *et al.* now describe in the current issue of the *Journal of Cell Biology*[1] the third mutant strain of *Dictyostelium* which lacks a major actin-binding protein and nevertheless shows normal motility and chemotaxis. The ground begins to shake beneath our feet! Could it be that, like those primitive people who believed that when the wind blows it is because the trees are stirring the air, we have the wrong end of the stick? Is it possible that cell locomotion is driven not by the cytoskeleton but by lipids, or something equally improbable?

The amoeboid form of *Dictyostelium discoideum* crawls in a very similar fashion to other animal cells and also shows a chemotactic response to distant sources of cyclic AMP. Unlike neutrophils and fibroblasts, however, these cells are haploid and therefore particularly suitable for genetic manipulation. Gerisch and his colleagues[2] at the Max Planck Institute in Martinsried in 1986 took advantage of this combination of virtues by isolating a mutant deficient in the actin crosslinking protein α-actinin. The group's more recent study[3] of this mutant, using four monoclonal antibodies, confirms that α-actinin is almost completely absent; nevertheless, the mutant's movements, chemotaxis and ability to 'cap' and 'patch' surface proteins are unimpaired.

An indication that this might be a general phenomenon came in two papers[4,5] published in the same issue of *Science* last year describing life without myosin. As related in a News and Views article at the time[6], strains of *D. discoideum* were generated that lacked normal high-molecular-weight myosin (myosin-2). In one case, expression of the myosin gene was severely depressed through the introduction of antisense RNA; in the other, homologous recombination was used to substitute a myosin lacking most of its tail for the normal myosin. It is something of a relief to relate that these myosin-less cells are not entirely normal: they have an altered morphology and are severely defective in their ability to carry out cytokinesis. But they can crawl and they can direct their locomotion up a gradient of cyclic AMP.

The point has now been driven home in the new work of Gerisch's group[1], in which André *et al.* describe a mutant strain of *Dictyostelium* lacking severin — an actin-filament fragmenting protein related to gelsolin and villin. The cells grow at the usual rate, forming normal fruiting bodies and viable spores. By many criteria, such as speed of movement, rate of turning and precision of chemotactic orientation, their motility is unimpaired.

A literal interpretation of these findings, based on conventional genetic arguments, would be that α-actinin, myosin and severin are not involved in cell locomotion. But this conclusion is at variance with much circumstantial evidence linking actin-based systems to the generation of motile structures in the cell. An alternative explanation is that these proteins could be involved in cell locomotion but their function could be duplicated or 'guaranteed' (as Schleicher *et al.* put it[3]) by more than a single protein. There is indeed substantial overlap in the activity of actin-binding proteins *in vitro* and more than one way to crosslink, fragment or even to move actin filaments. But why should there be such extensive 'fail-safe' guarantees for cell locomotion when other, more essential cellular functions such as DNA synthesis are not thus underwritten?

The explanation that we favour — which, if correct, has implications that extend beyond the area of cell motility — is that actin-based motility has some of the properties of the parallel distributed processes used in artificial intelligence (see Anderson's News and Views article[7]).

That is, at every stage between the detection of environmental cues by receptors on the cell surface to the generation of cell movement, there is extensive overlapping redundancy. Cell surface receptors change the levels of second messengers such as calcium ions and phosphatidyl inositol bisphosphate; second messengers influence the activity of actin-binding proteins, either directly or through protein kinases. At each successive stage, the 'signal' generated by a specific agonist becomes distributed more widely; it spreads down parallel pathways and converges with various combinations of signals from other receptors on to different target proteins.

The ten or more actin-binding proteins in a cell would form one layer of this net-

Paternity of dunnocks by DNA fingerprinting



W. B. Carr

THE hedge sparrow or dunnock (*Prunella modularis*) is a small, insect-feeding passerine bird belonging to the accentor family. It is common throughout the British Isles, inhabiting bushy places where nesting sites are plentiful and is a common sight in suburban parks and gardens. The species is unusual in having a variable mating system, a fact which has attracted considerable attention from behavioural ecologists. Mating may involve monogamy (one male, one female), polygyny (a male with two females), co-operative polyandry, where two males mate with a single female and may help to feed her young, and polygynandry, where two males share two or more females. On page 249 of this issue, T. Burke and collaborators report the successful application of the technique of DNA fingerprinting to link observations of mating behaviour and parental care of dunnocks in a well-studied population in a botanical garden in Cambridge, UK with precise measurements of reproductive success. They conclude that males do not discriminate between their own young and those of another male in multiply-sired broods, but feed offspring in relation to their access to the female during the mating period, which is a good predictor of paternity. Rory Howlett

in a set of different motile structures, each of which contains other proteins that perform similar functions. In this view, cell locomotion depends on the combined activities of actin-binding proteins as a whole (if Gerisch and his colleagues mutate all actin-binding proteins, the cell would grind to a halt). But it is a corporate exercise in which individual proteins have a range of functions that overlap the functions of other proteins. Removal of a single protein by mutation will not have a single identifiable phenotype but cause a 'graceful degradation' (a term used in parallel distributed processing) over a range of motile functions.

The analogy with parallel distributed processing networks may seem far-fetched, but it is no more than a recognition of the extensive interconnection in cell signals that is known to exist. The consequences of such overlaps have, simply, been most thoroughly explored in the case of computer networks used to simulate cognitive functions[8].

One important feature that has been found in such networks is that they are resistant to damage: as already mentioned, this feature is shared by the machinery of cell movement. Another is that individual units within the network come to represent regularities in the input: we might suppose, for example, that a rise in activity of a particular protein kinase represents all those sets of environmental conditions that signify 'round up' or 'divide'. Third, and of greater significance to evolution, is that parallel distributed networks are highly flexible in performance. Each type of cell movement could be driven by a specific 'blend' of actin-binding proteins ("take three ounces of actin; add three cups of filamin and two of severin; mix with a tablespoon of myosin and stir. . ."). And there are many more possible combinations of actin-binding proteins than there are individual proteins: an argument familiar to developmental biologists. □

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ARCHAEOASTRONOMY

Eclipse seen at ancient Ugarit

Christopher B. F. Walker

THE engraved tablet shown below is the only record of an arguably astronomical nature from the second millennium BC apart from those of Babylonia. Recovered from the site of Ugarit, an ancient city on the Mediterranean coast of Syria, it has been interpreted by Sawyer and Stephenson[1,2] as a report of a solar eclipse. On page 238 of this issue[3], de Jong and van Soldt deduce a new dating of this eclipse, to 1223 BC, basing their argument on archaeological considerations and a reassessment of the text. Their new dating is of great interest to those trying to calculate the change in speed of the Earth's rotation, but is inevitably speculative.

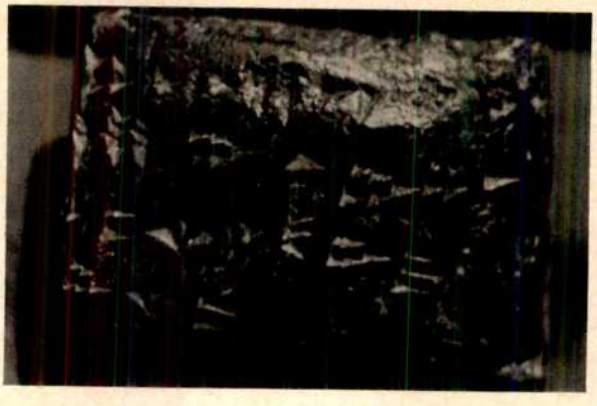
The publication and study of ancient and mediaeval astronomical records in recent decades has considerably increased the time base over which astronomical phenomena can be considered. The records contain references to eclipses, occultations, comets, sunspots and supernovae[4]. Babylonian[5] and Chinese[6] records have lately contributed to the study of the history of Halley's comet. The Babylonian 'astronomical diaries' from the period 400–50 BC[7] also contain records of market prices and weather reports which may eventually be useful for the reconstruction of economic and climatic patterns. Usable data of the same type from earlier than the seventh century BC are very rare.

The most important single group of observations has been those of the first and last visibility of Venus recorded during the reign of the Babylonian king Ammisaduqa (1702–1682 BC). Reconsideration of these in the light of later Babylonian data on the *arcus visionis* of the planet have led to a revision of the calculated date of the observations and consequently of the whole chronology of the Old Babylonian period[8]. The Ugaritic tablet is the only other arguably datable astronomical observation outside Babylonia from the second millennium BC.

In assessing the strength of the claims made[1–3] for the Ugaritic tablet, one must take into account the fact that this is, if correctly identified, the sole surviving astronomical observation written in the Ugaritic language. The only related material preserved in the palace archives of Ugarit are two fragments described as almanacs and an astrological text. Whereas Babylonian astronomical terminology, including the terminology of eclipses, is well known, we have no information on Ugaritic terminology. At first sight the text refers to an event occurring at sunset. Its interpretation as a non-technical description of a solar eclipse was suggested by Sawyer and Stephenson[1,2] on the basis of similar circumlocutory descriptions of eclipses ranging from China in the thirteenth century BC to Germany in the twelfth century AD. Corroboration of the interpretation depends on the planetary and calendrical evidence.

The identification of the Ugaritic god's name Rŝp with Mars and the proximity of Mars to the eclipsed Sun are critical to de Jong and van Soldt's analysis. The Ugaritic names for the planets are otherwise unknown, and the identification depends on the twice-attested equation of Rŝp with the Mesopotamian god Nergal and on a single Assyrian astrological omen text of the seventh century BC in which Mars, elsewhere always named Salbatanu, is unusually given the name Nergal. The normal usage in Mesopotamia was to give the specific names of the planets in observational references, and to introduce the names of associated deities only in astrological explanations. The use of the name Rŝp in the Ugaritic text would contrast with this pattern.

As de Jong and van Soldt make clear, their interpretation of the text as an eclipse report is also dependent on the assumption that a calendar of Egyptian



Mission Archeologique de Ras Shamra/Ugarit

Obverse side of the Ugaritic tablet KTU 1.78, presently in the Museum of Antiquities in Damascus. Arguably this describes a solar eclipse, dated by de Jong and van Soldt in this issue[3] to have occurred in 1223 BC. (Courtesy of D. Pardee.)

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type was in use. Until now there has been nothing to demonstrate whether Ugarit followed the Egyptian or the Babylonian pattern. Thus the present assumption, while perfectly plausible, will remain speculative until supporting evidence becomes available.

Given the paucity of contemporary material it is perhaps unlikely that we will ever find more explicit astronomical reports from Ugarit, and we have to make the best use of what we have. De Jong and van Soldt's work is certainly an advance on the earlier discussion. But the limitations of the evidence are such that at present their interpretation can best be regarded as a plausible hypothesis. To an Assyriologist it would seem to be a precarious basis for scientific arguments. □

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PICORNAVIRUSES

Finding the receptors

Stephen C. Harrison

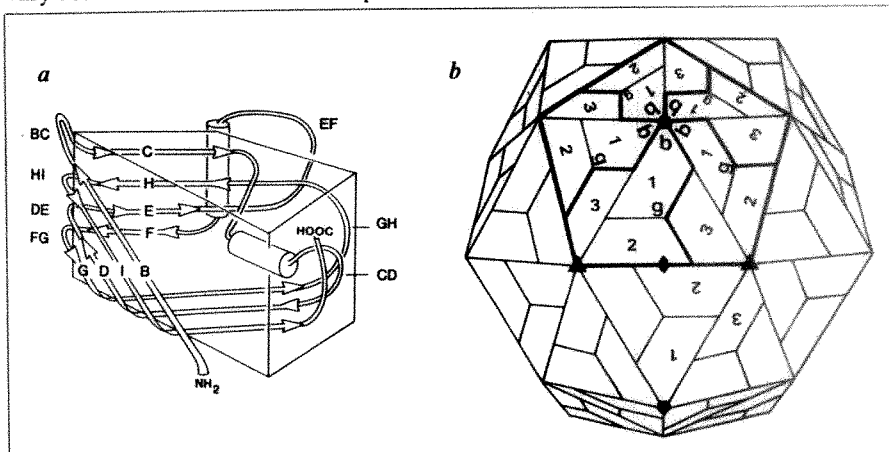
NEW insights and new puzzles about how RNA viruses recognize their receptors arise from the structure of foot-and-mouth disease virus, published three weeks ago in *Nature*[1], and from the identification of the receptors for human rhinoviruses and for poliovirus, reported this month in *Cell*[2-4]. The structure of the foot-and-mouth disease virus also provides a plausible explanation for the efficacy of a particular peptide vaccine.

Like other picornaviruses, foot-and-mouth disease virus (FMDV) is composed of 60 copies each of four polypeptides (VP1–4), arranged with icosahedral symmetry. Although not obviously related in sequence, VP1, 2 and 3 have similar folded structures, based on an eight-stranded β-barrel that I have elsewhere[5] proposed be called an RVC domain (for RNA virus capsid, where it was discovered) — see figure. The β-sheet framework is similar in the three subunits, but the loops and amino- and carboxy-terminal extensions vary considerably. VP4 is, in effect, part of the amino-terminal extension of VP2, from which it is cleaved during assembly. The human rhinovirus 14 (HRV14)[6] and poliovirus[7] structures show that the loops between framework elements dominate the surface of the virus and provide the important antigenic determinants.

In HRV14 and in poliovirus, VP1 contains particularly prominent BC and GH loops at opposite ends of the wedge-like barrel. These loops create a concave surface, and the packing of five copies of VP1 around the 5-fold symmetry axis means that these concavities join to form a roughly circular groove (see figure caption). This feature in HRV14 has been called the 'canyon' by Rossmann and colleagues[6]. These authors proposed that it might be the locus of receptor binding and noted that it would be inaccessible to antibodies. Colonno and colleagues have obtained evidence that the HRV14 canyon is its receptor site, by generation of site-directed mutants in infectious complementary DNA clones[8].

Several viruses show antigenic variation that permits escape from neutralization by antibodies present in a host previously exposed to a parent strain. Conserved receptor sites cannot undergo this variation. Must they be inaccessible to avoid neutralization by antibodies against their conserved structure? Colman has observed that a conserved receptor site need not compromise potential escape mutants, provided that the site is smaller than the typical 'footprint' of an antibody[9]. Variation at the periphery of the site can prevent neutralization without interfering with receptor interactions. Indeed, the influenza virus receptor site is a small, sialic acid-binding pocket of conserved amino acids surrounded by residues that vary between recurrent influenza epidemics[10]. The FMDV structure seems to show another kind of attachment site with similar properties. Several lines of evidence point to participation of the GH loop of VP1 in receptor binding[1,11]. Relatively short peptides with sequences from this loop block attachment of virus to cells[11]. They contain an evolutionarily conserved Arg-Gly-Asp sequence, which is surrounded by residues that vary from strain to strain. Not only does the GH loop project from the viral surface, it is also sufficiently disordered that it does not appear in the electron-density map. Thus, the presumed binding site in FMDV appears to be prominent, not recessed, but it is smaller than an immunoglobulin 'footprint' and embedded in a variable surround.

Potent vaccines can be made from peptides having sequences in the disordered GH loop of VP1[12]. The unusual success of these peptide vaccines can probably now be explained. Like a peptide in solution, the loop has no unique, folded conformation. A given antibody molecule that has been raised against a peptide antigen will be complementary to that peptide in a particular conformation. The corresponding sequence in the flexible loop can adopt the required conformation with little cost in free energy. Moreover, if different specificities in a serum recognize different conformations of the same peptide epitope, all will bind well to the virion.



a, The folded arrangement of the polypeptide chain in an RVC domain, as found in icosahedral, positive-strand RNA viruses. The framework strands of the eight-stranded β-barrel are denoted by letters B–I. The intervening loops and terminal extensions vary significantly among RNA viral proteins, but the size and shape of the framework is relatively constant. The upper surface of the domain in this diagram faces the outside of the particle when packed in the capsid of an RNA virus. The structure of tumour necrosis factor (TNF), a non-viral protein with a similar folded conformation, is described by Jones *et al.* on page 225 of this issue[25]. TNF looks very similar to a simple RVC domain, but its packing in a trimer involves different interfaces from those used by subunits of RNA viruses. Barrels of similar topology, but of rather different size and shape, are found in other viral and non-viral proteins. ***b***, The packing of RVC domains in an icosahedral viral shell. Some of the copies of VP1, 2 and 3 are labelled. There are 60 copies of each in the virus. VP4, cleaved from the amino terminus of VP2, is internal. The letters 'b' and 'g' show positions of the BC and GH loops in VP1. In FMDV, the BC loop of VP1 is truncated and the GH loop is disordered. The virus particle therefore has 'dimples' on its 5-fold symmetry axes and a flexible protrusion near the positions marked 'g' (and their symmetry equivalents). In HRV14 and in poliovirus, the BC and GH loops of VP1 are both well-ordered and prominent. The cavity between them forms part of a groove or canyon that circulates around the 5-fold axes (roughly joining the positions marked '1' in the upper part of the diagram).

Neutralization of picornaviruses by antibodies is believed to require some degree of 'crosslinking' between subunits, perhaps to inhibit an essential expansion or concerted rearrangement of proteins in the capsid[13,14]. The flexibility of the GH loop in VP1 of FMDV could facilitate such crosslinking. Correlations between disorder and response to antipeptide antisera have been noticed before[15,16]. The GH loop of FMDV is a particularly striking example and the first to be associated with a successful vaccine.

Cells of many types contain adhesion proteins — collectively called integrins — that recognize the Arg-Gly-Asp sequence in proteins in the extracellular matrix[17]. The inhibition of FMDV attachment by peptides containing Arg-Gly-Asp receptors suggests that one or more of the integrins is the FMDV receptor. The definitive identification of receptors for two other picornaviruses — human rhinovirus and poliovirus — implicates other kinds of adhesion proteins as viral binding targets. Two families of human rhinoviruses have been identified, recognizing distinct receptors[18]. The main group binds to a protein of relative molecular mass 90,000[19] that has now been identified in two laboratories as intercellular adhesion molecule-1 (ICAM-1), a surface protein found on many kinds of cells[2,3]. This molecule mediates leukocyte adhesion by binding to a protein called lymphocyte function-associated molecule-1 (LFA-1), a homologue of the integrins[20]. ICAM-1 itself is a member of the so-called immunoglobulin superfamily — proteins with domains similar to domains of the immunoglobulin molecule. It contains five concatenated immunoglobulin-like domains, a transmembrane segment and a short cytoplasmic tail[21,22]. The poliovirus receptor, also just identified, has a rather similar structure, with three rather than five immunoglobulin-like domains, but it seems to correspond to a previously unidentified member of the family[4].

Receptor specificity is generally regarded as a major determinant of cell and tissue tropism as well as of virus host range. In this regard, the distribution and expression of ICAM-1 and of the poliovirus receptor raise real questions. Both appear to be expressed in tissues that do not normally support growth of the virus that recognizes them[2-4]. Indeed, messenger RNA encoding the poliovirus receptor can be detected in cells to which virus does not even attach[4]. Yet the new papers show that when polio or HRV receptors are expressed in transfected mouse L cells, they confer ability to bind virus[2-4].

Adhesion molecules such as ICAM-1 and the integrins might seem to be odd candidates for viral receptors. Poliovirus and HRV 14 are believed to enter cells by the receptor-mediated endocytosis pathway[23]. Rapid endocytosis is not expected to be a property of matrix and intercellular-attachment proteins, however, and how these viruses are chaperoned into coated pits and endosomes now becomes a significant puzzle. Are adhesion molecules endocytosed when clustered (requiring polyvalency of viral attachment)? Or is attachment only the first in a more elaborate chain of events? In other ways, as the groups studying it point out, ICAM-1 is a highly suitable receptor for rhinoviruses. Its expression on epithelial and endothelial cells is significantly induced by soluble mediators of inflammation[24]. Therefore, a local virus infection may indirectly enhance the abundance of nearby receptors, through mechanisms of the host inflammatory response, or interfere with that response, by blocking ICAM-1. The more general notion, that in viral pathogenesis a virus–receptor interaction may do more than merely mediate attachment, is strengthened by these observations. □

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ENZYME CHEMISTRY

Designer catalytic antibodies

Robin J. Leatherbarrow

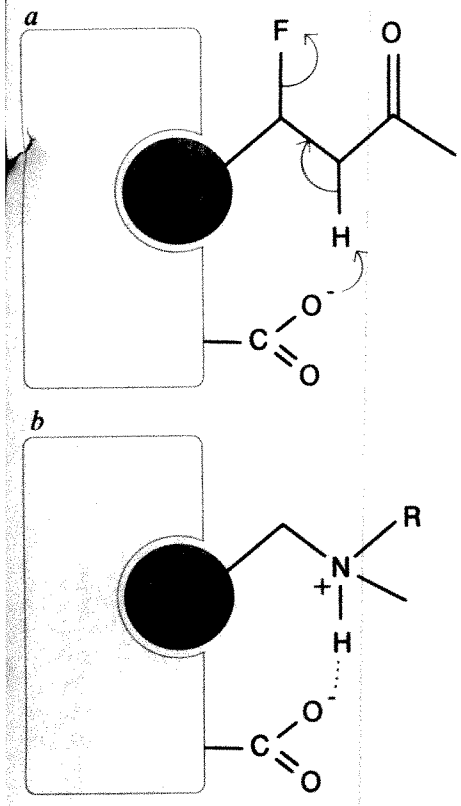
ENZYMES are well known to offer many advantages to the synthetic chemist. They often exhibit great specificity for the reaction catalysed, and work under mild conditions of temperature and pH in aqueous solution. The number of possible synthetic reactions, however, far exceeds the specificities of currently characterized enzymes, so there is considerable interest in producing enzymes with novel specificities by artifical means. Two routes have been used — protein engineering to alter the specificities of enzymes (ref. 1, for example); and the use of antibody molecules, which bind specific ligands (haptens) but which do not normally carry out chemical reactions. By clever design of the hapten it is possible to generate antibodies which catalyse specific reactions in the same way as an enzyme. On page 269 of this issue[2], Shokat *et al.* describe a new route to generate such catalytic antibodies.

Production of catalytic antibodies (abzymes) was first described in 1986 in simultaneous publications by the groups of Lerner[3] and of Schultz[4]. Their approach was based on two principles: that enzymes work by binding the transition state of a reaction better than the ground state; and that antibodies which bind specifically to any small molecule can be produced by coupling this molecule to a protein carrier and immunizing experimental animals. Lerner's and Schultz's groups reasoned that antibodies that were produced to bind transition-state analogues (molecules designed to mimic the shape and electronic configuration of the transition state) would function as enzymes towards the substrate of the reaction. Since then there has been considerable success in producing abzymes catalysing acyl-transfer reactions, carbon–carbon bond forming, and carbon–carbon bond cleaving reactions using these techniques (see ref. 2 and references therein).

Although the transition-state analogue approach has been successful, examination of natural enzymes shows that while some mechanisms involve exclusively transition-state stabilization[5], it is very common for groups within the active site to participate in the reaction. Acid/base catalysis involving proton donation or abstraction occurs particularly frequently. The problem addressed by Shokat *et al.*[2] is how such groups can be placed in the active site of an antibody with the precision required to generate catalytic activity. The reaction the authors chose was a β-elimination, reasoning that catalysis would be obtained by positioning a carboxyl group on the abzyme to abstract a proton. The desired reaction is shown in *a* in the figure. The abzyme needs a binding site for the main part of the substrate, and a carboxyl group which reacts as shown.

To produce this abzyme the authors immunized mice with a hapten containing a positively charged group, which they hoped would result in antibodies having a complementary negatively charged carboxyl group in the correct position to

PLANETARY ORBITS



a, Schematic representation of the reaction to be catalysed. The abzyme (light grey) requires a binding site for the substrate, together with a carboxylate group placed to carry out proton abstraction. *b*, The hapten used to generate the abzyme. A positively charged group is present so that the resulting antibody will tend to have a complementary negatively charged group involved in binding.

react (*b* in the figure). Of six monoclonal antibodies isolated, four accelerate the rate of β-elimination. This is a very high success rate, and suggests that the approach will be valuable in other reactions.

Given the mechanism of the reaction shown in *a*, one can speculate on the effect of introducing other charged groups into the abzyme. A positive charge near the fluoro group may be expected to enhance the polarization of the C–F bond and increase the rate of reaction. An additional carboxyl group (negatively charged) could be built into the hapten for this purpose, in an attempt to generate an appropriate abzyme. Depending upon the chirality of this carboxyl hapten, it may be possible to create abzymes specific for one or other of the enantiomeric forms of the fluoro substrate, and so confer stereospecificity on the reaction. □

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Emerging stability and chaos

Andrea Milani

HAVE the planets been on more or less the same orbits, with only quasiperiodic variations, since the birth of the Solar System? This problem of the stability of the Solar System has been investigated for more than 200 years. The problem becomes even more difficult when one asks whether it is possible to compute the orbits of the planets for any time span, or whether there is a predictability horizon[1] beyond which there is no way reliably to compute where the planets will be (or were)? It now seems that the second question will be the first to be answered fully. J. Laskar reports on page 237 of this issue[2] that the orbits of the inner planets are chaotic: any two orbits with nearby initial conditions diverge exponentially over timescales of 5 million years. Thus any computation spanning more than a few tens of millions of years (much less than the age of the Solar System) gives results that do not reliably apply to nearby orbits, and hence may not apply at all to the real Solar System whose initial conditions are derived from observations of finite accuracy.

Our ability to compute the long-term orbits of the planets has leapt forwards over the past 5 years, mostly because of the availability of supercomputers. The project LONGSTOP brought together several research groups (including my own) to compute the orbits of the outer planets (from Jupiter to Pluto) for 100 million years[3]. The Digital Orrery, a parallel computer specifically designed and built to solve *N*-body problems, has recently increased this limit to 845 million years[4].

Laskar uses a 'conventional' supercomputer, but exploits the dedicated computer algebra processors developed at the Bureau des Longitudes in Paris to perform analytical averaging of the equations of motion[5]; this permits the orbits of all the large planets, including the fast-moving Mercury, to be followed for 200 million years. When these figures are compared with the age of the Solar System (4.5×10^9 years), the solution of the problem of the stability of the Solar System might appear close — only one order of magnitude further away. But if the orbits are chaotic, the problem cannot be solved by computing just one orbit for the required time span.

In analytical theories of the planetary orbits a series representation of the solution is constructed, and so-called small divisors occur, as a result of the presence of a dense set of resonances between the fundamental frequencies. A resonance occurs when the ratio between two frequencies, or their combinations, is precisely the ratio of two integers. Even if the main resonances (with simple ratios such as 3:2) are avoided, the minor ones are always close. As a result, the number of terms that have to be taken into account grows too fast for such a theory to achieve the accuracy which would be needed, even with the most powerful computer algebra systems. This difficulty was known to Poincaré[6], who showed that any perturbation theory constructed to apply to a set of given initial conditions must give divergent series. Thus the use of supercomputers to perform numerical integrations is not a concession to fashion but the only possible way forward.

The next difficulty is encountered when the results of such numerical integrations become available. Although the series resulting from a purely analytical theory are divergent, there are solutions to the *N*-body problem that can be represented by means of a convergent Fourier series, namely the 'conditionally periodic' solutions found by Kolmogorov, Arnold and Moser (see ref. 7). In most of these there are few fundamental lines and a wealth of combination lines with rapidly decreasing amplitude.

If the solution of the initial-condition problem used to model the Solar System belongs to this class, then the numerically computed solution can be fitted to a conditionally periodic solution. When this technique of the synthetic theory[3] was used for the major outer planets, the LONGSTOP team[4] found that the fit was very poor. The behaviour seems not to be 'conditionally periodic', but rather consistent with a solution chaotically wandering in a thin region of the phase space. For the inner planets, Laskar[2] has found that a conditionally periodic model is impossible to fit, suggesting a quite large chaotic region.

To some specialists of dynamics, the evidence that an orbit is not a conditionally periodic solution is as good as proving that it is chaotic. Unfortunately, there is no rigorous proof that every orbit must be either conditionally periodic or chaotic, or a case of escape/collision, although there is no evidence for other possibilities at present. Direct proof of chaotic motion for some large planet is also needed. This can be obtained by computing two initially nearby orbits and monitoring the rate of increase of their separation; if the rate is exponential with time, the orbit is chaotic and the coefficient inside the exponential is the (maximum) Liapunov exponent. This was first achieved for the orbit of Pluto by the Digital Orrery team, which recently reported that two nearby Plutos diverge with a Liapunov exponent of 1 per

20 million years; moreover Pluto appears to have a continuous spectrum[4].

Laskar finds an even shorter timescale for the exponential separation of the orbits of the inner planets[2]. In principle, if one planet is in a chaotic orbit, then they all are. But whatever Pluto, Mercury and Mars do, the large outer planets are so little perturbed by them that in practice chaotic effects on the larger planets should occur with negligible amplitude. Thus we do not yet have direct evidence of, for example, Jupiter being in a significantly chaotic orbit, although this is expected to be the case, possibly with a somewhat smaller Liapunov exponent. It should be stressed that the precise values of the Liapunov exponents are extremely sensitive to small changes in the initial conditions, the planetary masses and the model used in the computation; thus the exact values might change as a result of future work. But presuming that the order of magnitude of the Liapunov exponents is correct as currently calculated, it is clear that the orbits of all the planets cannot be reliably computed for a time span as long as the age of the Solar System.

Chaos, however, does not necessarily imply that the system is unstable, that individual orbits will change drastically. Although instability might occur, the timescale over which this will happen is unknown. If the solutions are chaotic, then any statement of the behaviour of the planetary orbits must be of a probabilistic nature, and has to be investigated by computing many solutions with different initial conditions. Although this cannot yet be done, there is a preliminary task that is within reach of our present computing capability, namely to understand the qualitative features of these chaotic motions.

The interesting point is that in systems such as the Solar System — those that are only slightly perturbed from being integrable (analytically solvable) — chaos does not arise at random. Rather, it arises because of resonances, and the region of phase space sampled is shaped by those resonances. An investigation of the resonances in the Solar System should thus permit an approximation of the minimum size of the chaotic region and hence allow the prediction of the likelihood and extent of instability. The computation of the maximum size of the chaotic region is not yet possible: transitions from one resonance to another can occur, even if the two do not overlap, in exponentially long time spans which cannot be calculated except for simple cases.

This resonance analysis is now in progress for the two examples of proven chaotic motion described above — Pluto and the inner planets. An interesting feature seems to be emerging, namely the ubiquitous nature of secular resonance. This feature can be defined as a resonance in which the longitudes of the planets — with periods between a few months and a few hundred years — do not appear. Because the perihelia and nodes revolve with frequencies, denoted by g_k and s_k, respectively ($k = 1,\ldots,9$), corresponding to periods of tens of thousands of years, a combination frequency can be considered nearly resonant if its period is many millions of years.

Pluto is locked in three different resonances, one involving the longitudes of Pluto and Neptune; another an irregularity of the perihelion of Pluto; and a third, secular resonance recently discovered in our LONGSTOP data (*Icarus*, submitted). All these resonances appear to result in regular librations, however, and not to disrupt the stability of the orbit of Pluto. Another small divisor which might be relevant is $(g_9 - g_8) - 3(s_8 - s_9)$; this combination of the fundamental frequencies is essentially zero in the Digital Orrery solution, and significantly different from zero in the LONGSTOP solution. The cause of this discrepancy is the source of some controversy between the two teams, but clearly very small changes used in the computation, for example to the planetary masses, can result in displacement either inside or outside a resonance with a very small associated chaotic region. For the other outer planets the smallest divisor is $2(g_5 - g_7) + (s_7 - s_8)$, and again there is a suspicion of different behaviour in the different solutions so far computed. In his most recent, as yet unpublished, data, Laskar finds that the divisor $2(g_1 - g_3) + (s_3 - s_4)$ is the main cause of the large Liapunov exponent found for the inner planets. The similarity between these secular resonances is remarkable.

For the moment, we are left with the enigma of an orbit of Pluto locked in so many resonances, chaotic, and still ostensibly stable over timescales of hundreds of millions of years. As for the inner planets, they seem to be even more strongly chaotic, and nevertheless they are still here. The problem is now to understand how chaotic orbits can be stable for timescales much longer than the inverse of the Liapunov exponent. The results from the current generation of long-term orbit computations seem to indicate that this is the rule rather than the exception for planetary orbits. □

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DAEDALUS

New eyes for old

ALL of us, as we grow older, lose our ability to see closely in sharp focus. Bifocal, trifocal and even distributed-focal-length glasses are very imperfect solutions. Daedalus once invented a variable-focus pair of spectacles whose flexible plastic-film lenses could be pumped up with water to change the focal length — rather like the lens of the eye itself. He now returns to this idea with the crucial advance needed to make it practical and popular. His new variable-focus spectacles can focus themselves automatically.

After some thought, he has rejected the autofocus techniques used by modern cameras. His Autospecs do not measure the distance of the subject by means of an infrared beam; nor do they form an image and optimize its high spatial-frequency contrast. Instead, they take their cue from the eye itself. As the lens beneath the cornea focuses under the wearer's optical reflexes, its contour changes, and the cornea bulges or flattens in sympathy. Autospecs follow this process by means of small infrared sources on the spectacle frame, which bounce a pattern of beams off the surface of the eye to deduce its frontal curvature. The Autospecs then adjust their focus to aid the eye in its efforts. The whole arrangement acts as a fast-acting closed-loop servo system amplifying the wearer's own instinctive focusing. He will find his accommodation range magically extended. The proportion of focusing effort taken over by Autospecs will depend on their loop-gain. With sufficient loop-gain, they will restore focal power to almost fixed-focus eyes. As long as the wearer's instinctive effort to focus is perceptible at the cornea, the new glasses will sweep through their focal range until he is seeing sharply, and that effort relaxes.

This new technology will be widely welcomed. Bifocal glasses, which trade strained eyes for an arthritic neck, will rapidly vanish. Daedalus's prototype is rather heavy and clumsy, combining as it does corrective glass lenses, variable-focus liquid lenses, and quite a lot of electronics, but DREADCO's opticians are working hard to miniaturize it. They hope to give it a focal range far exceeding that of the eye itself, letting the wearer focus as closely and sharply as if he had a magnifying glass. Watchmakers, instrument mechanics, stamp-collectors and entomologists will be especially grateful. Daedalus even hopes for large sales among the young, who can focus quite well unaided. With his Autospecs focusing for him, the lenses of a youthful wearer will not be fatigued and work-hardened over the years by constant flexing. He will enter his middle years with eyes so wonderfully youthful that they may never lose their natural accommodation at all.

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Distemper virus in Baikal seals

SIR—An acute disease of Lake Baikal seals (*Phoca sibirica*) attributable to morbillivirus infection became evident in the autumn of 1987 when weakened seals crawled onto the lake's icy shores and died. Many of them had paralysed hind extremities and ophthalmitis. No similar event is known from records going back to the 1930s. By October 1988, several thousand of the 80,000–100,000 seals in Lake Baikal had died.

In December 1987, during an expedition to Ushkani Island on the border between middle and northern Baikal, one of us (V.S.K.) noticed that three of the five dogs that lived there and had close contact with seals had died with typical symptoms of canine distemper virus (CDV) infection.

In the same month, we observed signs of a similar disease in a 6-year-old female seal (code PB-6). Two days after capture the animal seemed to have recovered but after a further 3 days it again showed symptoms of acute disease — diarrhoea, ophthalmitis and convulsions of the hind flippers. It died 10 days after capture. Rectal temperature varied between 34 °C on days 1 and 10, and 24 °C on days 5–7. No severe pathology of internal organs was observed but cytological investigations of bladder epithelium showed intracytoplasmic eosinophilic inclusion bodies characteristics of canine distemper[1]. Inclusions were also found in cells of liver, kidneys, spleen, lungs and in neurons of the brain and spinal cord.

When the sera of eight dead seals were studied, six gave weakly positive indirect haemagglutination reactions (titres of 1:10 to 1:80) with measles antigen[2], which is a close relative of CDV; all sera neutralized the cytopathic effect of CDV vaccine strain EPM[3] with titres from 1:8 to 1:64.

To prove the presence of a CDV-like virus in seal tissues, we have recently used the technique of oligonucleotide probing. On the basis of the known sequences of CDV RNA[4,5] we synthesized 14–40 deoxyoligonucleotide probes complementary to either virion RNA, or to the corresponding messenger RNA. The probes were designed to detect sequences that are either common to CDV and measles virus (probes 1–7, for gene *N* sequences, and probes 14–19 for gene *P* sequences), or sequences that differ between the two viruses (probes 8–13 for the gene *N* sequences that differ most). The precise sequences probed are shown in *a* in the figure. Probing revealed both virion and mRNA sequences in seal PB-6. These data suggest that CDV or a close relative was not only present but biologically active, inducing synthesis of its mRNA. These results and the preliminary immunological data were discussed at a conference held in Irkutsk in February 1988.

In June 1988, several hundred samples of blood and tissues of Lake Baikal seals were obtained during the spring cull. Of 83 spleen samples subjected to oligonucleotide probing, 8% gave a strong positive signal for CDV RNA and there was a total of 55% positive samples. The 45% negative samples confirmed that the assay is selective (*b* in the figure).

Along with CDV-neutralization tests, we also performed both radioimmunometric assays, using CDV vaccine absorbed on nitrocellulose and rabbit anti-seal IgG labelled with ^{125}I, and enzyme-linked immunosorbent assays (ELISA), using a monolayer of Vero cells infected with CDV in plastic wells and protein A conjugated with peroxidase[6]. A large proportion of animals tested contained antibodies against CDV in their blood.

In the neutralization assay, 55% of the sera had titres between 1:16 and 1:64; 50% were positive in the radioimmunometric assay; and 89% were positive by ELISA, with titres from 1:16 to 1:1,280. These data indicated that the whole population of Lake Baikal seals had been in contact with the virus, suggesting that the survivors should be immune to further infections. The lack of any unusual mortality of seals in the autumn or winter of 1988 confirms that immunity is now established.

We mentioned[7] CDV as the cause of the Baikal seal disease in September 1988 when, first from newspapers, and then from Dr A. Osterhaus, we learned that CDV or a closely related virus was also believed to have caused the disease of seals in Western Europe. This has been confirmed by several techniques[8,9]. According to nucleic acid hybridization and immunological data[10,11] the virus in European seals is not identical to CDV but a distinct seal virus (phocid distemper virus; PDV). Our data on Baikal seals prove only that they were infected by a morbillivirus closely related to CDV; its precise relationship to other morbilliviruses will only be established when the seal virus RNA has been sequenced. The fact that seals in Baikal and in Western Europe have been infected by very similar, or identical, viruses poses new questions concerning the origin and spread of the seal virus.

During a very recent visit, Dr A. Osterhaus brought us sera of European seals, some of which contained antibodies (with titres up to 1:1,080) to CDV in our ELISA. These data and those reported below by Osterhaus *et al.* strongly suggest that the Baikal and European seals have been infected by the same virus, or one that is closely related.

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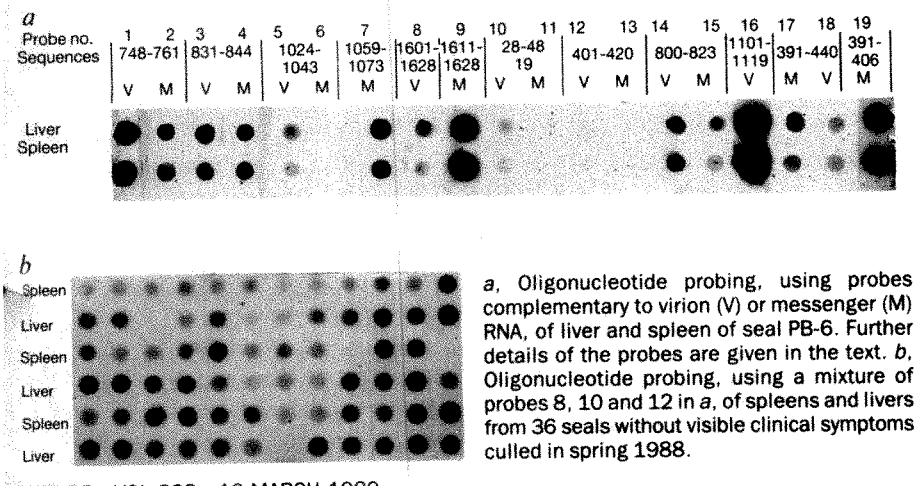
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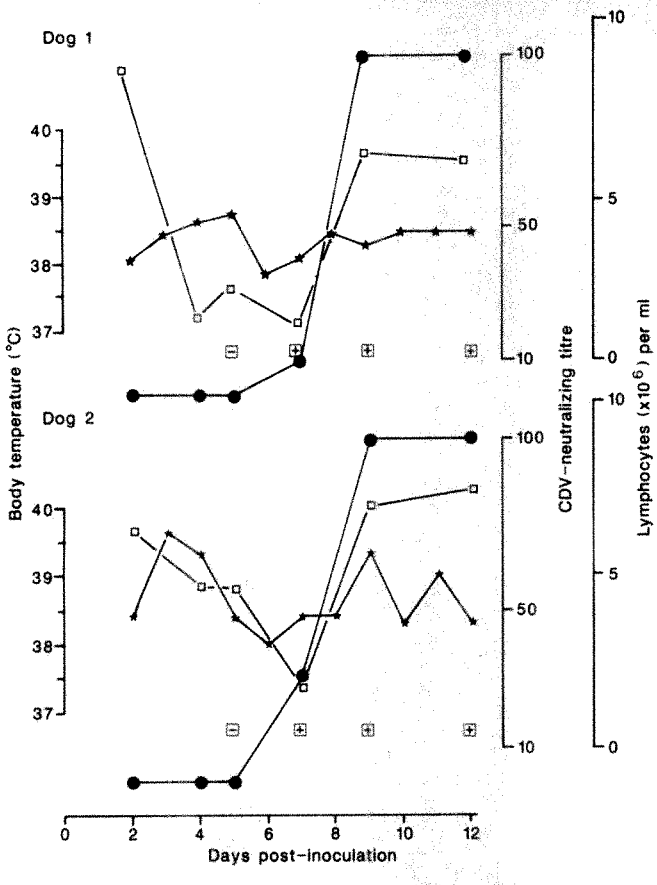
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SIR—We have demonstrated the presence of an infectious morbillivirus in the organs of a Baikal seal (*Phoca sibirica*) collected on a recent visit to Lake Baikal.

The seal was one of three that had suffered from a canine distemper-like disease,



a, Oligonucleotide probing, using probes complementary to virion (V) or messenger (M) RNA, of liver and spleen of seal PB-6. Further details of the probes are given in the text. *b*, Oligonucleotide probing, using a mixture of probes 8, 10 and 12 in *a*, of spleens and livers from 36 seals without visible clinical symptoms culled in spring 1988.



Body temperature (*), lymphocyte counts (□), development of CDV-neutralizing antibodies (●) and virus isolation (⊞) during 12 days after inoculation of a suspension of liver and spleen from a Baikal seal into two dogs.

had the typical histopathological lesions of the disease in harbour seals (*P. vitulina*), and whose serum contained canine distemper virus (CDV)-neutralizing antibodies. Samples (2 ml) of a 10 per cent mixture of liver and spleen suspension were inoculated into two 12-week-old specific pathogen-free Beagle dogs, as previously described[9]. The animals were observed for the development of clinical symptoms and tested for the development of CDV-neutralizing and ELISA antibodies in their sera, for 12 days. As shown in the figure, both dogs developed essentially the same mild clinical symptoms as dogs inoculated with material from the European harbour seals, including a slight body temperature rise and lymphopaenia within 1 week, and mild respiratory symptoms including mucopurulent nasal discharge within 2 weeks. Antibodies were detectable within 7 days, and the presence of a morbillivirus in peripheral blood mononuclear cells of both dogs on days 7, 9 and 12 post-inoculation was demonstrated as previously described in the above reference.

These data clearly confirm earlier speculations that a morbillivirus identical with, or similar to, the virus that struck northwest European harbour seals, had previously infected and caused canine distemper-like symptoms in Baikal seals. We hope to compare the biological and molecular properties of the morbilliviruses isolated from the Siberian and European outbreaks in an attempt to discover whether there was an epizootiological link between the two outbreaks. If so, the morbillivirus may have spread from Siberia to Europe within 6 months either by terrestrial carnivores or by means of the extensive bird migration between Siberia and Europe.

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Time asymmetry

SIR—Wolpert's reservations[1] on the 'Brussels School' approach to the problem of irreversibility, as summarized in Coveney's review[2], require a response.

Wolpert asks "why the underlying (time-symmetric) physics should imply the (overtly time-asymmetric) equations"? It is now well understood that it is precisely the instability of the underlying conservative reversible dynamics, which leads to the realization of the unitary group evolution, as a semigroup breaking the temporal symmetry.

As an example, let us consider the Kolmogorov systems, also mentioned in Coveney's review. These systems are characterized by a stable and an unstable partition of the initial data into cells evolving asymmetrically in time, under the conservative dynamical evolution. The remarkable fact about these partitions is that they are self-propagated by the dynamics, and not destroyed, as ordinary coarse graining partitions would be. The cells of the stable or K partition become refined in the future and coarser in the past, whereas the cells of the unstable partition experience the reverse behaviour under the dynamical evolution.

Therefore, for Kolmogorov systems an arrow of time is intrinsically built in, by observing the time-asymmetric evolution of the partitions. For these systems the second law is expressed as a selection principle of initial conditions. Only initial data leading to equilibrium in the future may be realized. The selection principle of the future arrow corresponds to a non-unitary realization of dynamics as a Markov process. This realization results from the operational limitations due to the continuous refinement under the dynamical evolution, to controlling or distinguishing trajectories in the same cell of the stable K partition. For these reasons K systems have been called intrinsically irreversible[3,4]. Therefore, Wolpert's first requirement — that the underlying dynamics be reversible — is automatically satisfied for the conservative K systems.

Concerning Wolpert's second requirement "why nature in fact chooses one direction in time over the other", for K systems there exist two semigroups, one leading to equilibrium in the future and the other in the past. The choice of the semigroup leading to equilibrium in our future corresponds to our physical experience. It is the basis of the formulation of the second law of thermodynamics: entropy increases in the direction of our future. The existence of a unique priviledged direction of time for all processes in our observable universe refers to the compatibility of the future arrow of intrinsically irreversible dynamical systems with the cosmological arrow of time. (For a recent discussion of the cosmological

roblem see ref 5.)

Wolpert returns to old criticisms of Boltzmann's pioneering work. In view of he recent developments of unstable lynamical systems it is now well under-tood[6] that the objections raised against Boltzmann, such as the Zermelo paradox, ire not meaningful, as the Poincaré recur-ence time is much larger than the Liapunov time, being the time after which ve lose control over the trajectories of a lynamical system.

In short, recent developments, inclu-ling those of the Brussels School, permit one to incorporate the second law into the formulation of dynamics for highly unsta-ble dynamical systems. In this way, the seeming contradiction between the rever-sibility of dynamics and the irreversibility of the second law has been overcome.

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'Amylin' hormone

SIR—Leighton and Cooper[1] recently reported a potential physiological function of a newly discovered pancreatic β-cell hormone which they refer to as 'amylin'. The introduction of the term amylin is a source of potential confusion because this peptide has also been called islet amyloid polypeptide (IAPP)[2-4] and diabetes-associated peptide (DAP)[5]. This fact prompts us also to point out recent progress in relating IAPP/DAP/amylin, which we prefer to call IAPP, to type-2 diabetes (age-related diabetes mellitus).

The 37-amino-acid IAPP is the main component of amyloid deposits of the islets of Langerhans occurring in association with type-2 diabetes in humans and cats[2-5]. It is a normal product of pancreatic β-cells[3,6,7] that is colocalized and probably cosecreted with insulin[8,9] and generated through proteolytic processing of an 89-amino-acid precursor (ref. 10; C. Betsholtz *et al.*, unpublished data).

IAPP inhibits insulin-stimulated glycogen synthesis in skeletal muscle preparations *in vitro*[1]. This is interesting in relation to the pathogenesis of type-2 diabetes because this disease is characterized by peripheral insulin resistance in addition to islet dysfunction.

That amyloid formation is of importance to the pathogenesis of type-2 diabetes is implied by the correlation between the presence of an amino-acid motif in the IAPP molecule and both amyloid formation and the spontaneous onset of type-2 diabetes in adult humans, cats and racoons; this motif is lacking in species that do not develop islet amyloid or type-2 diabetes (C. Betsholtz *et al.*, unpublished data).

Thus, changes in IAPP synthesis, processing or release may relate to both major traits of the type-2 diabetic syndrome; islet dysfunction through amyloid formation and insulin resistance through a peripheral action.

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On the SCIDs?

SIR—We recently reported (*Nature* **335**, 256–259; 1988) that a functional human immune system could be established effectively in mice with severe combined immunodeficiency (SCID mice) by inoculating them intraperitoneally with peripheral blood leukocytes (PBL) of human origin. The major immunological conclusions of that study are, first that recipient mice show prolonged survival with no ill effects of graft-versus-host disease; second, that the human leukocytes, including both B and T lymphocytes, increase in number and survive in these mice now for at least one year; third, that the reconstituted mice show significant levels of spontaneously secreted human immunoglobulin; and, last, that specific human antibody responses are inducible by immunization. These have all been confirmed in subsequent studies involving many more PBL-reconstituted mice.

More recent studies, however, have indicated that the numbers of intraperitoneally inoculated human PBL that migrate to the spleen, lymph nodes and peripheral blood during the first five-week period after reconstitution may be significantly less than those reported in Fig. 3 of our paper. For reasons not yet fully understood, antibodies used for detection of human cells show high levels of non-specific binding to splenic cells in these mice soon after reconstitution. Nevertheless, human cells do appear in these lymphoid organs in larger numbers at later times.

We caution other workers to be alert for this staining artefact, and we regret any inconvenience that our overestimates may have caused.

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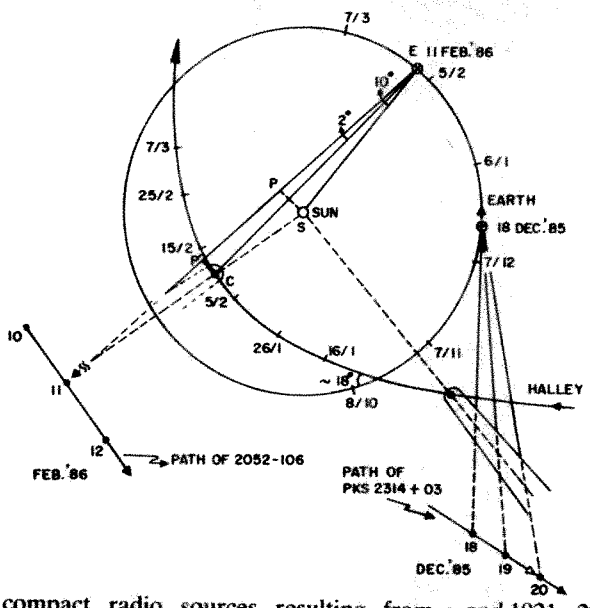
Quasar enhanced

SIR—During the recent appearance of Halley's comet, observations were reported[1,2] of enhanced scintillations of quasars occulted by the cometary ion-tail; however, Ananthakrishnan *et al.*[3] dispute such an origin for these scintillations.

The geometry at the time of both the 103-MHz observations[1] on 18–20 December 1985, and those at 327 MHz (ref. 3) on 10–12 February 1986, is depicted in the figure. On 18 December 1985, the source, PKS2314+03, lay at a solar elongation of about 85°, such that the solar wind contributed only to background scintillations, with an average scintillating flux of about 3 Jy (ref. 1). The observed enhancement was six times this value and 1.5 times the maximum flux normally caused by the solar wind, which, for 103 MHz, occurs at a solar elongation of about 30°.

During the 327-MHz observations[3] on 11 February 1986, the source 2052–106 was presumed to be occulted by the cometary tail. As the perihelion occurred on 9 February, the period 10–12 February is most inappropriate for observing such events: the solar elongation of 2052–106 on 11 February was about 10°, for which, at 327 MHz, strong scattering prevails. The scintillation index is near its maximum at around 14°. Consequently, most of the scattering occurs in a thin layer of the solar plasma centred around the point of closest approach to the line of sight from the Sun. The three control sources were also within 12° of the Sun. Therefore, the scintillation enhancements of all of the sources were due solely to the solar plasma. The source also gets broadened by strong scattering close to the Sun and enhanced outflow of cometary plasma, which results in reduced scintillation. These conditions are therefore unsuited to observing enhancement of scintillations by Halley's comet.

Thus, enhanced scintillations of



Relative positions of the Sun, the Earth and Halley's comet during the observations reported in refs 1 (December 1985) and 3 (February 1986). The paths of the quasar sources are also shown.

compact radio sources resulting from occultation by strong cometary ion-tails can be identified only for favourable alignments of the comet, Sun and observer. Such conditions existed during the observations reported in ref. 1 and it is possible, therefore, that the scintillations were of cometary origin. Unambiguous confirmation could be obtained by using more control sources close to the line of sight[4].

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ANANTHAKRISHNAN *ET AL.* REPLY—The importance of our letter[3] lies in its demonstration that control sources are essential in investigations of scintillation through the cometary plasma tail. Alurkar *et al.* also agree that control observations are essential. Without such observations it is difficult to determine whether or not the observed[1] enhancement in scintillation is caused by the plasma tail.

We disagree with the suggestion that because our observations of 2052−106 on 11 February 1986 were made at a solar elongation of about 10° (actually 11.2°), the circumstances were unsuitable for studying the enhancement of turbulence. On the contrary, the outflow of plasma from the comet would be an order of magnitude higher for our observations than for those of Alurkar *et al.*[1], made at a solar elongation of 85° and before perihelion. Furthermore, angular broadening effects are unimportant unless the source is very much closer to the Sun. Therefore, the 60% enhancement seen by us (Fig. 2 of ref. 1) could have arisen either from the comet or from the solar wind. However, a similar increase in the control source implies that a solar-wind origin is most probable. In addition, observations far from the Sun, of the sources 1817−391 and 1921−293 along with control sources, also show no enhancement resulting from the plasma tail[3].

We agree that geometry of occultation is important. Unfortunately, cometary occultations of the required geometry are extremely rare, and consequently there is a need to take every available future opportunity for making occultation observations of radio sources by cometary tails (along with suitable control sources) in order to resolve the present conflict of results.

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Zinc and LTP

SIR—Recent studies have implicated *N*-methyl-D-aspartate (NMDA) receptor activation and concurrent postsynaptic depolarization in the induction of long-term potentiation (LTP)[1]. Kauer *et al.*[2] recently showed that NMDA receptor activation produced only a transient potentiation in the CA1 region of the hippocampus and speculated that some additional factor, perhaps co-released with glutamate from presynaptic terminals, was essential for the later, long-lasting phase of LTP. We suggest that synaptic Zn^{2+} is the factor. Zn^{2+} is systematically localized in synaptic vesicles of excitatory synapses throughout the mammalian telencephalon[3] and probably is co-released with glutamate into the synaptic cleft with neuronal activity[4,5], especially at higher rates of stimulation Zn^{2+} decreases NMDA recepto mediated responses but increase quisqualate receptor-mediated respor ses[7,8], and may acutely modulate th postsynaptic response to glutamate. If th synaptically released Zn^{2+} can gain entr into postsynaptic neurons, it is possibl also that it could induce LTP. It has bee shown *in vitro*, that Zn^{2+} can activat protein kinase C and induce its transloca tion to membranes[9], both associated wit establishing LTP[1,2]. Entry of Zn^{2+} int neurons has not been clearly documente but it could pass through the NMDA channels. Zn^{2+} can block the NMDA channel (C. W. Christine and D. W. Choi manuscript in preparation). Mg^{2+}, an perhaps divalent cations in general, ma permeate the NMDA channel at a rat determined by the ease with whic surrounding water molecules can b lost[10,11]. Zinc can shed its water molecule at a rate between that of Ca^{2+} and magne sium[12], suggesting that its permeabilit may be greater than that of Mg^{2+}. The neurotoxicity of Zn^{2+} on cortical neurons is consistent with Zn^{2+} entry through the NMDA channel, as it is competitively blocked by the NMDA-channel blocker, MK-801, and increased by removal of extracellular Na^+ and calcium[13].

Thus the simple co-occurrence of NMDA receptor activation with post-synaptic depolarization could result in a calcium influx and transient postsynaptic potentiation. If the presynaptic neuron is firing sufficiently rapidly, Zn^{2+} could be released from presynaptic terminals and enter the postsynaptic neuron via NMDA channels, activating protein kinase C (and perhaps other intracellular processes) resulting in LTP.

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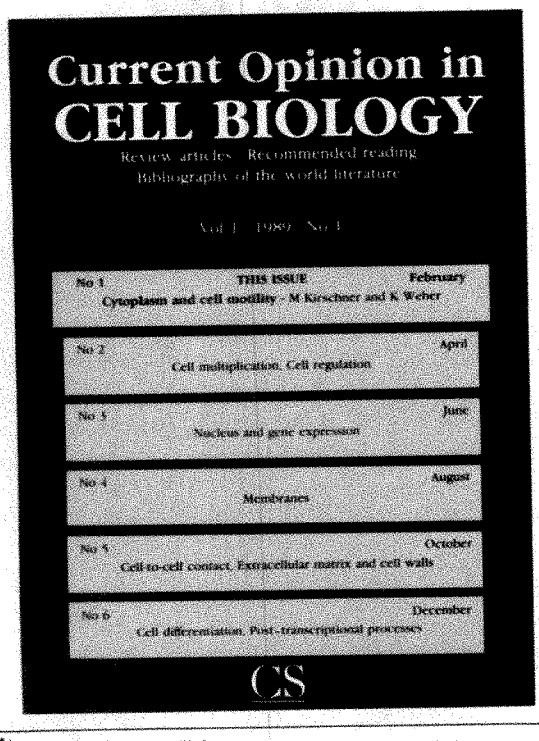
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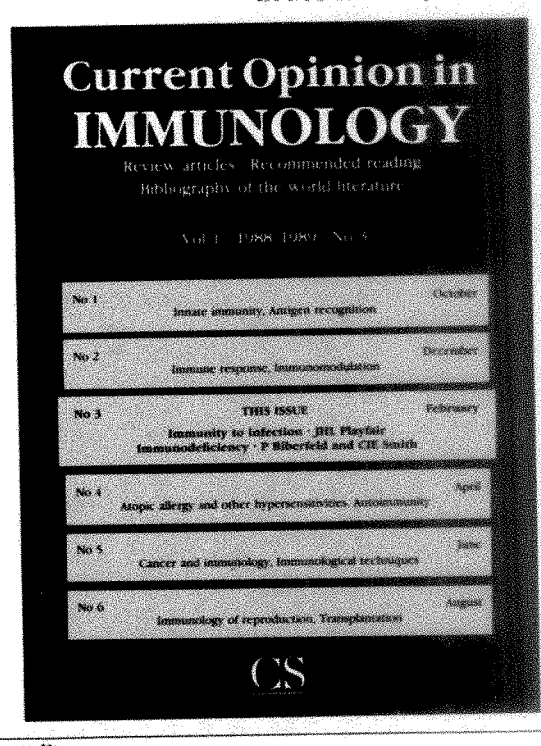
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The drug-reaction drama

Louis Lasagna

Responsibility for Drug-Induced Injury. By M.N.G. Dukes and D. Swartz. *Elsevier: 1988. Pp.431. Dfl.350, $184.25.*

RARELY does a new book have no competitor; Dukes and Swartz have written such a book. The authors' backgrounds are complementary, combining science (including political science) and the law. Although the literature on drug-related injury is abundant, as is that on the litigious implications thereof, Dukes and Swartz concentrate here on the *responsibility* for drug-induced injury. The volume should enjoy brisk sales, at least among lawyers and employees of pharmaceutical companies.

As the opening paragraph of the introduction (after a somewhat pompous first sentence) points out, it is important to determine when and how a drug injury is preventable, and in this assessment we need "to find out what went wrong". Unfortunately, that is often much more easily said than done. The 'Elixir Sulfanilamide' story is the exception; the deaths it caused were easy to explain and could all have been prevented, had the toxic solvent only been tested in animals for safety. Consider, in contrast, the Japanese 'S.M.O.N.' story, a devastating epidemic of neurological disease, blamed on clioquinol, a drug sold widely all over the world without manifesting toxicity like that seen in Japan. Even in retrospect, clioquinol seems likely to have been at best a 'co-villain', perhaps facilitating damage to the central nervous system by some neurotropic virus. And what of thalidomide, with which 'seal babies' could be reproduced in animals only with great difficulty after the fact, with many species, breeds and strains of rabbits, mice, rats, hamsters, dogs and primates failing to show the dreaded anomalies?

Dukes and Swartz say that "several thousand asthmatic patients may have been killed by high dose isoprenaline aerosols". But these high-dose preparations have disappeared, and 'epidemics' of asthma deaths have occurred periodically since then in different countries — only now they are generally attributed to undertreatment (for example with corticosteroids), not drug toxicity. Even with drugs taken off the market (benoxaprofen and Indosmos, for instance), the numbers cited for deaths attributable to them are soft indeed; sick, elderly patients who die while on a drug are not necessarily victims of the medication.

In an important introductory paragraph (p.6), the authors point out that in some countries (the United States being a prime example), there has been a tendency to seek compensation for injury from "the wealthiest or best-insured party (often the pharmaceutical industry) even when this has entailed redefining such considerations as foreseeability or retrospectively postulating the existence of a duty not previously recognized". Bendectin (Debendox) was the only prescription drug ever approved in the United States for treating the nausea and vomiting of pregnancy. Lawsuits alleging that it was a teratogen forced its withdrawal from the marketplace worldwide, even though there have been repeated verdicts of 'innocent' from expert panels and courts. One can safely wager that no sponsor will ever again try to market a drug for this indication in the United States (even though an early Australian critic of the drug has admitted faking his data). Fears of litigation have in effect driven most large US companies out of vaccine research and development, despite the clear need for new vaccines both for civilian and military populations.



Dukes has criticized 'me-too' drugs that "were merely slight variants on existing molecules . . . which provided no new benefit but merely introduced new risk". But it must be a rare pharmaceutical company that purposely markets inferior variants in today's very competitive market. Furthermore, half of the World Health Organization's *List of Essential Drugs* is made up of compounds that were *not* the first drugs in their therapeutic categories; many of these could be dubbed 'minor chemical modifications' of the breakthrough products. Would we really want to be restricted today to the earliest penicillins or the first sulphonamides?

The book accurately describes the complex basic problems in establishing causality, but does not adequately communicate the fact that certainty about the cause of a drug reaction (or even that one has occurred) is uncommon. Experts shown the data about a suspected reaction quite typically disagree vigorously — not least because multiple medications are often being taken by patients, and it is often impossible to rule out spontaneous co-morbidity unrelated to drugs, or the possibility that the patient is manifesting another aspect of his basic illness. One cannot usually state categorically that an ingested drug could *not* have caused a given adverse effect, but it is only rarely possible to state that a given drug was unquestionably the cause. Algorithms to identify causality (an area which my colleagues and I pioneered) are useful primarily as check lists, not as ways to eliminate uncertainty.

This book is not easy reading, and there are variations in the size of type that are difficult to interpret (is small for less-important material?). But potentially 'responsible' parties will ignore it at their peril. The authors address the obligations of a long list of players in the drug-reaction drama: physicians, dentists, medical students, manufacturers, drug retailers, dispensing pharmacists, nurses, patients, governments and official agencies. (One wishes that the legal profession were sometimes more 'responsible'; trivial and capricious suits are by no means uncommon — at least in the United States.)

Dukes and Swartz dedicate their opus to the World Health Organization. One can question, however, their assertion that "materials emanating from the WHO are probably the most authoritative of all documents on drug issues, representing a broad consensus". In my own experience with WHO committees, I have been struck by the compromises made necessary by the fact that the representatives from different countries must hammer out a document with which they can live on their return home. Consensus based on politics is not necessarily the ultimate in scientific authority. □

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Ways of being catty

Juliet Clutton-Brock

The Domestic Cat: The Biology of Its Behaviour. Edited by Dennis C. Turner and Patrick Bateson. *Cambridge University Press:1989. Pp.222. Hbk £35, $59.50; pbk £15, $22.95.*

IT IS probably because domestic mammals have been held in a certain disdain by zoologists that studies of their behaviour have been so neglected. To observe the behavioural patterns of the leopard, in the wild, will enhance status; it requires large sums of money, complicated equipment and personal courage; to watch the behaviour of the domestic cat requires only time, patience and experience in interpretation, yet the results may yield a greater knowledge of felid behaviour. Perhaps more importantly, they will yield information on the relationships between human beings and their animal companions, a subject that has been submerged for too long in pejorative terms such as 'anthropomorphism' and 'pet-keeping'.

This symposium volume, edited by Turner and Bateson, is the first work to bring together much of the research that has been carried out, in recent years, on the biological basis of behaviour in the domestic cat. It includes reports of studies on reproduction, social life, predatory behaviour, feral cats and house cats, all carried out with exemplary attention to the codes of ethology.

One aspect of research on the cat that is missing from the book is the connection between the patination and colour of the coat and temperament (as distinct from the link between breed and temperament which is discussed by Mendl and Harcourt, p.48). Karsh (p.173), when asking what influenced people's choice of a cat, was told by one woman that the personality of her cat was all-important to her. However, she later claimed not to be able to relate to a cat that was all white or all black, and that she related better to a cat that was grey or striped. Perhaps experience enabled her to predict what the general temperament of the animal would be from its pelage. In his article "Cats and Commerce" (*Scientific American* **237**, 100–107; 1977), Todd has postulated that black cats may be less aggressive and more tolerant of crowding than striped tabbies, which might therefore have a more distinctive temperament. Clearly, there is a need for more work on this topic.

An underlying thread in the book is the need to quantify the characteristics of individuality, and perhaps the greatest contribution to ethology that can be made from domestic animals will come from studies of behavioural variation. For unless a population of wild animals has been habituated, it is very difficult to categorize individual responses. People who live with cats know them as individuals; Mendl and Harcourt (pp.41–54) have made a start to defining individual variation and the biological significance of behavioural style.

The Domestic Cat is sure to become a classic in the study of behaviour in domestic animals. □

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- *Infinite in All Directions* by Freeman Dyson (Bessie/Harper & Row, New York). Price is $8.95. For review see *Nature* **332**, 748 (1988).
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- *Schrödinger: Centenary Celebration of a Polymath* edited by C. W. Kilmister (Cambridge University Press). Price is £12.50, $22.95. For review see *Nature* **326**, 913 (1987).

Out in the open

J.R.S. Fincham

The Release of Genetically-Engineered Micro-Organisms. Edited by M. Sussman, C.H. Collins, F.A. Skinner and D.E. Stewart-Tull. *Academic:1988. Pp.306. £29.50, $59.50.*

THE molecular cloning of genes and their transfer between different kinds of cells and organisms is now a standard analytical method, practised in almost every modern university biological department, and filling more than half of the pages of the leading biological journals. Anxiety about its safety was quite intense in some quarters in the late 1970s, but has largely died away.

Now we are faced with another wave of concern, this time arising from a wide range of schemes for putting genetically manipulated organisms to use outside the laboratory. Typically, this issue has generated less heat in Britain than in either the United States or continental Europe. This may, perhaps, have been a factor in the choice of Cardiff as the venue for the symposium from which this book stems. Sponsored jointly by the International Union of Microbiological Societies and three of its British and American constituents, it drew together contributions from a selection of the relevant university and institute-based scientists; scientists working in commercial laboratories were less well represented. The published proceedings include 13 papers, and about 100 pages are devoted to rapporteurs' accounts of eight round-table discussions.

The volume was hurried into print a mere seven months or so after the conference, and it suffers from most of the defects that make symposium proceedings (with a few distinguished exceptions) the most ephemeral and least worthwhile of library purchases. It lacks both the coherence of style and argument of a good book and the rigorous presentation of primary data that one expects in a good journal. The articles are uneven in style, ranging from the unduly terse to the prolix. The round-table reports contain some of the most interesting information, but it tends to be reduced to the status of anecdote by the lack of references. The index is very inadequate.

Nonetheless, this particular symposium volume is likely to attract much interest as a source of up-to-date information about the kinds of environmental release that are in prospect and the purposes that they are intended to serve. The symposium was mainly concerned with three areas of application: agriculture (improved nitrogen fixation and better pest control); biodegradation of chemical wastes; and the development of safe live vaccines. There is only one article (by J.E. Davies) on the present and impending applications to chemical and pharmaceutical industry. Important as these undoubtedly are, they do not involve environmental release but rather scaled-up indoor containment.

A theme running through several of the contributions is the need to ensure that any introduced micro-organism does not persist indefinitely in the environment. Roy Curtiss III proposes various non-lethal mutations, such as deficiency in cyclic AMP, which may militate effectively against long-term survival of engineered bacteria. D.H.L. Bishop *et al.*, in tests of a baculovirus intended for use as a narrow host-range insecticide against a moth, found that a mutant unable to make the polyhedral viral coat was still infectious but did not persist in the soil or on plant surfaces. It is intended, by further engineering, to make the virus even more deleterious to the moth than it is already, but this aspect is not dealt with in the book.

The main point of the article by S.E. Lindow and N.J. Panopoulos is that the now famous ice-minus *Pseudomonas syringae* does not survive well in the soil. Studies on persistence of released bacteria depend, of course, on efficient methods for detection and monitoring, the theme of the article by R.R. Colwell *et al.* Enumeration of soil bacteria by colony

counts depends on having specific selectable markers. Antibiotic resistance genes are generally unacceptable for environmental release but the *E. coli lacZ/Y* genes, which lend themselves to easy visual detection, are shown by D.J. Drahos *et al.* to provide a useful alternative. The most sensitive method of detection is probably to probe for a DNA sequence specific to the target organism after amplification of that sequence in the sample under test by the polymerase chain reaction. This does not, however, give a quantitative measure of abundance prior to amplification.

The promise of human benefit emerges most clearly in the articles on the development of new vaccines. A.A. Lindberg deals with vaccines against oral and enteric pathogenic bacteria, and E. Paoletti and his associates with the use of fowlpox virus vectors to vaccinate non-avian species. In one of the round-table discussions, E. Wedman reports vaccination of calves against Sinbis virus (not, in fact, a pathogen) with a recombinant between Sinbis and vaccinia.

There is nothing on the use of vaccinia virus as a vector for the expression of genes encoding immunogenic proteins of pathogens, except for a full documentation of the unhappy affair of the rabies vaccine trial, carried out on cattle in Argentina. The perfectly respectable sponsors of the trial had failed to obtain clearance from the local authorities, and incurred universal denunciation. Clearly, innovators in this area need to have both a proper regard for safety and acute political sensitivity. Live vaccines produced by genetic engineering, though likely to be safer than the old vaccines obtained by attenuation of pathogenic viruses and brought into use on a trial-and-error basis, will doubtless have to satisfy far more stringent safety criteria before being accepted.

Those seeking controversy will be disappointed in this volume. Although there is preoccupation with containment, nobody puts forward any scenario of disaster should containment fail. There are a number of rather coy references to 'perceived' risks, but the perceivers do not appear to have been present at the symposium. Although many of the public expressions of concern have undoubtedly been irrational or perhaps (as in the furore about the ice-minus bacteria) merely symbolic, it is difficult to take efficient precautions if one does not have some idea of what one is guarding against. No doubt the scientists present did have their own perceptions of hazard, or lack of it. It would have made for a better-balanced symposium had these been more explicitly discussed. □

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Notes from the quantum field

Kate Metropolis & Chris Quigg

Beamtimes and Lifetimes: The World of High Energy Physicists. By Sharon Traweek. *Harvard University Press: 1988. Pp. 187. $20, £15.95.*

IF A coprophagous coleopteran stopped to reflect, its life would no doubt seem a noble quest. Nevertheless, the attention lavished upon the dung beetle by a Serious Entomologist would be flattering in the extreme, applauding — by reason of the entomologist's devoted interest — this scarab's life work. So it is with high-energy physicists and anthropologists: the life of the mind, the quest for nature's secrets, is suffused with its own divinity. But surely, to be taken seriously by serious persons must be the sincerest form of flattery. Sharon Traweek is a Serious Anthropologist and our colleagues must needs be flattered by her fascination with their habits and tribal customs.

The community Professor Traweek has chosen to study, that of experimental high-energy physicists, seems apt for classical anthropological analysis. The group has a shared past, anticipates a shared future, has rites for initiating new members and is able to distinguish itself from other communities. The population is divided into highly ordered, long-lasting associations: the handful of high-energy accelerator laboratories comprise several groups, each executing an experiment in its own style. A group consists of 50 to a few hundred physicists from different institutions, with varying backgrounds, specialities and levels of experience — graduate students, postdoctoral fellows, professors, laboratory staff members — who build a detector to gather data on subatomic particles and then analyse the information to construct a more complete picture of the Universe. Subgroups, sometimes individuals, are responsible for different parts of the hardware, software and analysis. Although people occasionally move between experiments and laboratories, the collaboration may last for a decade or more. The society is highly ordered, rich in mythology and customs; and, because there are communities of experimental high-energy physicists around the world, it offers anthropologists the opportunity to distinguish behaviour patterns endemic in the profession from expressions of national or ethnic traits.

Particle physics has well-known personalities — clowns, tyrants, thoughtful moralists, egomaniacs, wizards — about whom many people are curious. Are they typical? How do they interact? What order underlies a group in which the list of workers on a single experiment may fill an entire page of a journal? An analysis that revealed the strengths and weaknesses of a particular approach to large collaborations could be interesting, amusing and even cautionary for scientists in fields beginning to collectivize. Indeed, Traweek comes upon provocative questions: What are the norms of behaviour within the community? How are actions and motives analysed? Why do people who see each other day in, day out recount different versions of history?

Scientific inquiry requires that researchers play Devil's Advocate until they can convince themselves that their data are complete and can be trusted. Unlike the physicists she studies, Traweek evinces no scepticism towards her data and fails to convince us that she has gathered anything more than an idiosyncratic sample. *Beamtimes and Lifetimes* reads less like a mature analysis than like a collection of undigested field notes recording observations at SLAC, the Stanford Linear Accelerator Center in the United States, and KEK, the national high-energy physics laboratory in Japan.

Traweek consistently uses the experience or opinions of a few individuals to draw broad conclusions about the entire community. If these illustrations were used to vitalize an observation or conclusion, they would be useful. Without a convincing richness of supporting data, however, her inductions have a foundation of quicksand. Further, her decision not to identify her subjects means that they become interchangeable: one group leader, one laboratory director is presented as indistinguishable from another. Yet in fact these people have quite different personal and scientific styles. She does not make a plausible case for not differentiating them, so the reader can only conclude that her powers of observation are not equal to her task.

This judgement is reinforced by Traweek's methodology. Reporting a discussion of how wives of high-energy physicists supported their husbands' work, she writes, "The older wife of a laboratory director interjected that she thought the way to avoid distracting the husband from his important work was to pursue one's own interests seriously. By their glances at each other I gathered that the younger women disagreed". A moment's reflection provides a number of alternative interpretations: interest in each other's reaction to an unfamiliar point of view, exasperation over hearing this position put forth for the millionth time by someone in a situation different from theirs, and so forth.

A recurring theme for Traweek is gender bias and sexism in the education, the rites of passage and the practice of experimental high-energy physics — a subject that should not be trivialized. If

particle physics *is* a male culture (although saying so doesn't make it so), it is important to understand how it came to be that way and why it remains true. Traweek's summation is as cogent as the evidence that Beatles records, played backwards, contain messages of devil worship:

> The language used by physicists about and around detectors is genital: the imagery of the names SPEAR, SLAC, and PEP is clear, as is the reference to the "beam" as "up" or "down." One must see the magnets at LASS to appreciate the labial associations in the detector's name, Large Aperture Solenoid Spectrometer. Ironically, the denial of human agency in the construction of science coexists with the imaging of scientists as male and nature as female. Detectors are the site of their coupling: standing on the massive, throbbing body of the eighty-two-inch bubble chamber at SLAC while watching the accelerated particles from the beam collide twice a second with superheated hydrogen molecules made this clear to me.

The plethora of facts that Traweek has got wrong does nothing to encourage faith in her inductions. Descriptions of scientific apparatus, events and phenomena, intended to confer verisimilitude, are grossly inaccurate. Her judgements are no more convincing: the statement that the food at SLAC is "rather good, for a cafeteria" will come as a shock to anyone who has eaten there.

The neglect of the reasons that inspire people to join and remain in this community is a crippling omission. Particle physics is not a closed or isolated society in which membership is determined by happenstance. Because the only motive Traweek recognizes is career advancement, her narrative is oblivious to the excitement, the exhilaration of doing physics. Nowhere do we see people driven by curiosity, intoxicated with the creation and understanding of new experience.

Beamtimes and Lifetimes is not a complete or reliable guide to the community of particle physicists. Let it be taken nevertheless as an invitation to particle physicists to re-examine their values, institutions and assumptions about each other and about the rest of the world. □

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Life transformed

Adrian Desmond

The Age of Lamarck: Evolutionary Theories in France 1790–1830. By Pietro Corsi. Translated by Jonathan Mandelbaum. *University of California Press: 1988. Pp. 360. $42.*

AT THE entrance to the Jardin des Plantes, opposite the Seine, stands an imposing statue of Lamarck, the "Founder of the Doctrine of Evolution". And in relief, at the back, is a touching scene depicting a lonely, dejected Lamarck being consoled by his sister. This image, of an isolated trailblazing figure, is surely one of the most enduring in the history of biology.

Now Pietro Corsi, in his stunning study of Parisian science after the Revolution, shatters this myth. He questions the long-standing assumption that Lamarck was a prophetic genius, striding alone, out of time and place. The truth was very different; so much so that Corsi actually talks of an 'evolutionary culture', of which Lamarck was but a part. And in investigating this culture, he has given us the most compelling account yet of French transformism between 1800 and 1830.

Historians have been quick to deny Lamarck an audience. But Corsi argues that the eighteenth-century buffonian tradition — with its grand, unifying view of nature and attempt to look at life through time — still had a populist appeal, even after the Revolution. And it was to the buffonians (most by then outside academia) that Lamarck spoke — to the marginal men appalled by the increasing specialization of science. They deplored Cuvier's attempt to subordinate natural history to a descriptive, analytical comparative anatomy. There were still plenty of these outsiders in 1800, criticizing Cuvier for failing to investigate the larger laws of nature. It was against this cultural background that Lamarck's own ambitiously theoretical works, with their rival approaches to *biologie* (his word), were formulated.

Lamarck started out as a botanist, chemist and conchologist, and he was appointed the professor of insects and worms at the Muséum d'Histoire Naturelle in 1793. Before 1800 he remained a staunch anti-transformist, adamant too that life and inorganic matter were separated by a chasm. Then suddenly in 1800 he switched tracks. His subsequent evolutionary views have always been difficult to interpret (if easy to parody). Corsi explains the origin and real meaning of Lamarck's ideas by tracing their development in detail through the critical period 1800–1802. By this means he is also able to show that, far from accepting spontaneous generation first, as historians had thought, Lamarck only warmed to it after shifting to evolution.

The fact that Lamarck rarely quoted or cited fellow authors has led to the belief that he was acting alone. But again Corsi introduces us to numerous other dissident intellectuals at loggerheads with the powerful Cuvier. Many rejected Cuvier's claim that some fossil animals were extinct, perhaps killed off by a marine inundation. Like Lamarck, they believed that these animals had simply changed their form in a gradually changing world. As a result, Corsi is able to show that Lamarck's famous *Philosophie Zoologique* (1809) was less an insular pioneering work than a running dialogue with his contemporaries.

Given this context, Lamarck's science makes sense. Of course there were inconsistencies. Lamarck had inordinate trouble squaring his progressing animal series with his non-progressionist geology; but, by the 1820s, a new generation had cast aside his old framework and mated evolution to a directionalist Earth history, which provided the environmental motor. There was also a good deal of frustration with the man himself: Lamarck exasperated even sympathizers with his pig-headedness, and they were less and less inclined to come to his aid. Even so, as Corsi says, he was never totally deserted by his friends, any more than he was completely ignored by his enemies, the way the old histories implied.



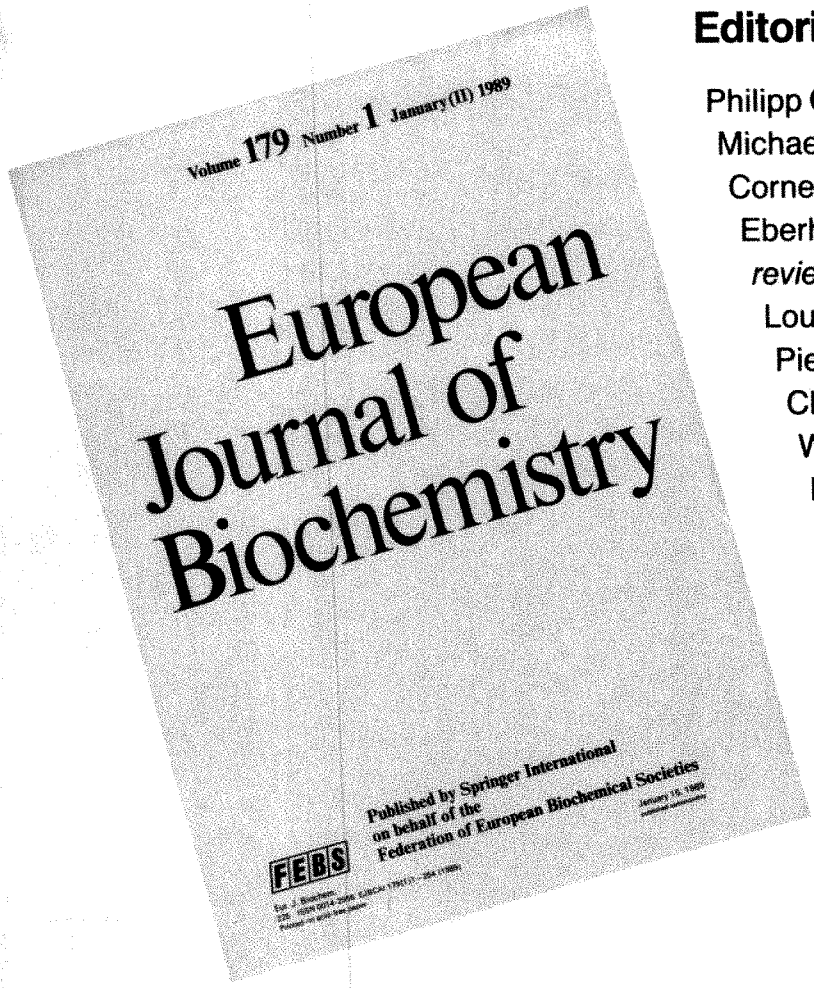
Still thinking — Lamarck's statue at the entrance to the Jardin des Plantes in Paris.

The Age of Lamarck was first published in Italy in 1983, and this translation (excellent in itself) has been well worth waiting for. Corsi makes Lamarck a man of his time, and that is no mean feat. For historians the result is a treat. But for Lamarck — pilloried and parodied for two centuries — it is long overdue justice. □

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RNA evolution and the origins of life

Gerald F. Joyce

The evolution of RNA is likely to have played an important role in the very early history of life on Earth but it is doubtful that life began with RNA. Consideration of what came before RNA must take into account relevant information from geochemistry, prebiotic chemistry and nucleic acid biochemistry.

THE question of life's origins is one of the oldest and most difficult in biology. The answer, if it is ever known, will not be a single statement of fact but rather an extended chronology, beginning with the formation of the Earth and ending with the appearance of cellular organisms. The problem is especially difficult because we have no direct evidence of the events that occurred during roughly the first thousand million years of the Earth's history. The oldest rocks that provide clues to life's distant past are 3.6×10^9 years old and by that time cellular life seems already to have been well established[1]. Modern organisms are so sophisticated that they furnish little information about what life was like before there was a genetic code and a translation apparatus. Extraterrestrial studies have yet to provide us with an alternative life form for comparison. We are left with only a partial understanding of the origins of life that is based largely on inference and conjecture.

From time to time in the quest for such partial answers, a new experimental fact emerges that forces us to view the problem of the origins of life in a different light. The results of the classical Miller–Urey experiments, in which amino acids were produced by circulating H_2, CH_4, NH_3 and H_2O past an electric discharge[2], for example, make it hard to escape the conclusion that amino acids were present on the prebiotic Earth. The synthesis of purines by polymerization of HCN (ref. 3) similarly suggests that adenine, guanine and other purines were available. The most recent example of such a finding is the discovery of RNA molecules with catalytic activity[4,5]: in addition to their recognized properties as templates, RNA molecules have enzymatic properties and are thus the only molecules known to function as both genotype and phenotype.

This has led to a revival of interest in the idea that there was a time, before the origin of protein synthesis, when life was based entirely on RNA. The idea is not new: it dates back eighteen years before the discovery of catalytic RNA[6] and was discussed extensively in the late 1960s[7–9]. But the discovery of catalytic RNA caused molecular biologists to begin to take the notion of an 'RNA world' seriously[10–12]. It is by no means a foregone conclusion that life began with the RNA world, although several lines of evidence suggest that the RNA world existed at some stage in evolutionary development. Here, I shall briefly review the advantages of RNA as the basis for the early evolution of life, before discussing the chemical obstacles to the initiation of an RNA world and suggesting how in principle they might have been overcome.

Why RNA?

A fundamental property of living systems is that they have a chemical basis for the storage of genetic information. RNA, DNA and other nucleic-acid-like compounds are informational macromolecules that have inherent template properties and so lend themselves in a straightforward way to both storing information and replicating it. It has been argued that to focus on nucleic acids is to take too parochial a view: there may have been other information storage systems with template properties that were more readily available on the primitive Earth. Cairns-Smith, for example, has proposed[13] that life began with self-replicating clays, with the genetic information stored as a distribution of charges along a clay surface and replicated by ionic interactions between existing and newly-formed surface layers. Other templating systems have been proposed based on organic compounds other than nucleic acids[14,15] or a combination of minerals and associated organics[16]. While keeping an open mind about these possibilities, enthusiasm has to be tempered until there is experimental evidence that such an entity can both replicate and code for specific catalytic behaviours.

Could it be that the first genetic molecule did not have template properties and replicated itself in some other way? There was a time when it was widely believed that life began with self-replicating proteins[17]. After all, polypeptides are capable of a wide range of catalytic behaviours and it is very likely that they were produced abiotically on the primitive Earth[18]. The amino-acid sequence of abiotic polypeptides would have been tremendously heterogeneous, so we must postulate that some mechanism existed to ensure that a particularly useful sequence would facilitate the production of additional copies of itself. Dyson first suggested the possibility of a self-sustaining network of proteins in which the components mutually catalyse the synthesis of each other from monomeric starting materials[19]. Kaufmann has argued that, given a large enough assemblage of random polypeptides, the emergence of a self-sustaining network is almost inevitable[20]. This conclusion seems to me to rest on a highly over-optimistic estimate of the probability that a random polypeptide can catalyse peptide-bond formation with any significant degree of sequence specificity. Any experimental evidence to the contrary would be wecome.

The evidence that life was based on RNA at some stage in evolutionary history is circumstantial but, I believe, persuasive. None of the following points is convincing by itself but together they constitute a body of evidence that cannot be dismissed as coincidental. First, the template properties of RNA enormously simplify the task of self-replication. RNA genomes are known to exist in certain viruses and Orgel has shown that RNA templates are able to direct the synthesis of complementary oligomers in a chemical system[21]. Second, RNA has catalytic properties, as exemplified by the self-splicing activity of certain group I and group II introns[4,22], the transfer RNA processing activity of the RNA component of RNase P (ref. 5), and the self-cleaving activity of 'hammerheads' and related RNA structures[23,24]. These behaviours, although of doubtful prebiotic significance in themselves, indicate that life based on RNA would have included function as well as informational properties. Third, RNA plays an important part in modern biological systems, appearing most conspicuously in those cellular processes that are presumed to be among the most ancient. RNA primes DNA synthesis during DNA replication, carries the genetic message from DNA to the translation apparatus, gathers and positions amino acids in accordance with the codon structure of messenger RNA and functions as an integral part of the ribosome. RNA also has a central role in the spliceosome apparatus[25,26] and is an essential component of the ribonucleoprotein enzyme that directs telomere synthesis in eukaryotic cells[27]. Fourth, most of the biological coenyzmes are nucleotides or compounds that could be derived from nucleotides (Table 1), a curious fact that may indicate that RNA and RNA enzymes were present before the development of protein synthesis[28]. Fifth, histidine, which is important in enzyme catalysis as either

a nucleophile or general acid–base catalyst, is produced by a very unusual biosynthetic pathway that begins with phosphoribosyl pyrophosphate and adenosine triphosphate (ATP). The functional imidazole moiety, which now operates as part of a histidine residue within a protein enzyme may once have operated as part of a purine base within an RNA enzyme[28]. Sixth, in contemporary metabolism, deoxyribonucleotides are synthesized by enzymatic reduction of ribonucleotides rather than by a biosynthetic pathway comparable to that used to synthesize ribonucleotides. The most widely distributed reaction system makes use of three nucleotide-derived components: two molecules of flavin adenine dinucleotide (FAD) associated with thioredoxin, and one molecule of nicotinamide adenine dinucleotide phosphate (NADP). Seventh, deoxythymidylic acid is formed by 5-methylation of deoxyuridylic acid, again suggesting the primacy of ribonucleosides over deoxyribonucleosides.

The most important feature of RNA is that it combines genotype and phenotype in a single molecule, so that replication of RNA enables darwinian evolution to occur at the molecular level. Genetic variation is introduced as a result of mutational errors and RNA-catalysed recombination events. This variation is in turn expressed as a range of phenotypes. Because of differences in their chemical behaviour, some RNAs will replicate more efficiently than others and will grow to dominate the population until a new variant with even greater selective advantage arises[29]. This is the RNA world.

There are several objections to the notion that life began with RNA, that fall into two general categories. First, RNA is biochemically inept, particularly when compared to proteins whose catalytic prowess is so apparent in modern biochemistry. With the discovery of catalytic RNA, this argument has lost some force but it can still be said that RNA does not seem as versatile a catalyst as protein is. Second, RNA is not a plausible prebiotic

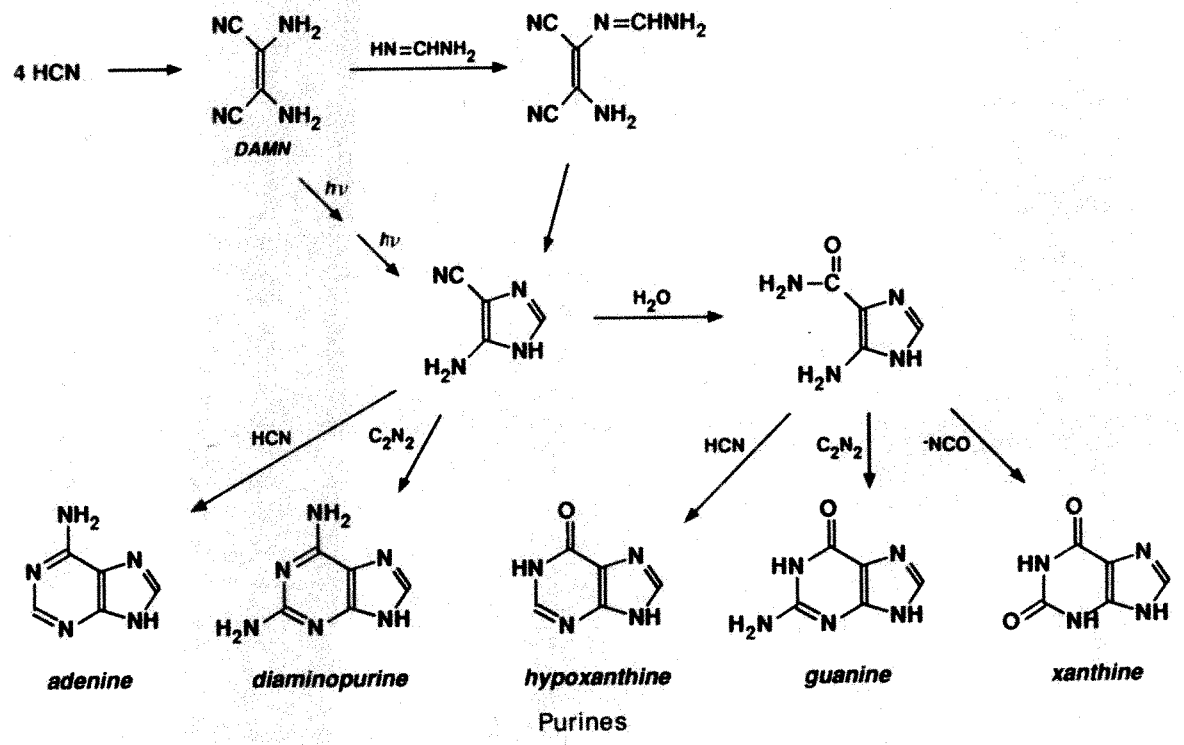


FIG. 1 Prebiotic synthesis of purines by self-condensation of HCN. HCN undergoes a tetramerization reaction to produce diaminomaleonitrile (DAMN). The rate-determining step is the attack on HCN by CN^- to form a dimer, rapidly converted to a trimer and then to the tetramer, DAMN. This reacts with either formamidine or ultraviolet light to produce 4-aminoimidazole-5-carbonitrile, which in turn leads to the formation of various purines.

TABLE 1 Coenzymes that resemble nucleotides

Coenzyme	Base	Sugar–phosphate	Substituent
NAD	Adenine	Ribose 5′-phosphate; ribose 5′-phosphate	Nicotinamide
NADP	Adenine	Ribose 2′, 5′-bisphosphate; ribose 5′-phosphate	Nicotinamide
FAD	Adenine	Ribose 5′-phosphate; ribitol 5′-phosphate	Isoalloxazine
FMN	—	Ribitol 5′-phosphate	Isoalloxazine
TPP	Pyrimidine	Pyrophosphate	Hydroxyethylthiazole
THF	2-Amino-4-hydroxy-6-methylpteridine	—	*p*-aminobenzoic acid; glutamic acid
Coenzyme A	Adenine	Ribose 3′-phosphate, 5′-diphosphate	Pantothenic acid; β-mercaptoethylamine
Coenzyme B_{12}	Adenine; 5,6-dimethyl-benzimidazole	5′-Deoxyribose; α-ribose 3′-phosphate	Cobalamin
S-adenosylmethionine	Adenine	Ribose	Methionine
ATP	Adenine	Ribose 5′-triphosphate	—
UDP-glucose	Uracil	Ribose 5′-diphosphate	Glucose
CDP-diacylglycerol	Cytosine	Ribose 5′-diphosphate	Diacylglycerol

The carrier molecules *S*-adenosylmethionine, ATP, UDP-glucose and CDP-diacylglycerol are included with the traditional coenzymes. A number of other nucleoside diphosphate sugars are known to act as carriers in contemporary metabolism. FMN, flavin mononucleotide; TPP, thiamine pyrophosphate; THF tetrahydrofolate; UDP, uridine diphosphate; CDP, cytidine diphosphate.

molecule because it is unlikely to have been produced in significant quantities on the primitive Earth[30]. This is a more serious criticism that goes to the heart of the question of whether RNA was the first genetic molecule or whether it took on the role of genetic material at a later stage in evolutionary history. Next, therefore, I consider how difficult it would have been to synthesize RNA in the prebiotic environment.

The primordial setting

Perhaps the most surprising aspect of the origins of life on Earth, other than the fact it happened at all, is that it happened so quickly. The Earth was formed from a diffuse cloud of cosmic gas and dust about 4.6×10^9 years ago[31] and by most accounts, was a very inhospitable place for the first $0.4–0.6 \times 10^9$ years of its history. It is virtually certain that no useful organic chemistry could have occurred until about $4.0–4.2 \times 10^9$ years ago—after the Earth's crust had formed[32] and the level of meteoric impacts had subsided so that the environment was no longer in constant upheaval[33]. On the other hand, the fossil record strongly supports the existence of cellular life by 3.6×10^9 years ago[34] and it has been claimed that indirect evidence exists for biological carbon fixation as early as 3.8×10^9 years ago[35]. The time window for biogenesis, from organic chemistry to a primitive cell, seems to be 'only' about 0.4×10^9 years.

The composition of the prebiotic atmosphere is a problem of fundamental importance to our understanding of the origins of life. Until recently, it was generally thought that the prebiotic atmosphere was strongly reducing, containing mostly H_2, CH_4, NH_3 and H_2O[36]. As scientific models of planetary formation have become more sophisticated, it has become increasingly clear that we do not really know whether the prebiotic atmosphere was strongly reducing, mildly reducing or even non-reducing, although it seems certain that there was no free oxygen ($<10^{-6}$ atmospheres) until the advent of oxygen-producing photosynthetic bacteria about $2.5–2.9 \times 10^9$ years ago[37]. At present, the weight of evidence indicates that the prebiotic atmosphere was not strongly reducing.

The atmosphere was formed primarily by outgassing of volatile components of the primitive mantle. Its initial composition depends on whether outgassing occurred before or after the iron-rich core of the planet had formed: if outgassing occurred before core formation, contact with metallic iron would have ensured a strongly reducing atmosphere, containing mostly H_2, H_2O, CH_4 and CO; if the iron had already sunk to the core, the redox state would have been determined by the Fe^{2+}/Fe^{3+} ratio in iron minerals, resulting in a less reducing atmosphere that contained H_2O, CO_2, H_2 and CO but almost no CH_4[38]. It has been argued that even if reduced species such as CH_4 and NH_3 were produced before core formation, they would have been rapidly depleted by photodecomposition[39]. As time went on, escape of H_2 from the upper atmosphere and outgassing of less reduced species would have tipped the chemical balance in favour of CO and CO_2 over CH_4 and in favour of N_2 over NH_3. There may have been some mechanism for the continued production of CH_4 and NH_3, such as the slow re-equilibration of CO_2 and CH_4 at temperatures below 873K (ref. 40), by the arrival of CH_4 and NH_3 on comets and meteorites[41] or by the photochemical reduction of CO_2 in the presence of Fe^{2+} (ref. 42).

Although a strongly reducing environment is most favourable for the synthesis of organic compounds, several prebiotic syntheses have been demonstrated under less strongly reducing conditions. Consider the synthesis of hydrogen cyanide and formaldehyde, which in turn provide a good starting point for the synthesis of more complex materials. Both HCN and H_2CO have been obtained in good yield in spark-discharge experiments under mildly reducing conditions (partial pressure of N_2 of 100 torr and H_2 of 0–400 torr)[43]. Even in the absence of H_2, the yield of HCN and H_2CO is about 2% (based on carbon) or about 2–3 nmol J^{-1} (based on the energy input). This works out to about 4.5×10^9 kg yr^{-1} on the primitive Earth, assuming the energy available from the solar corona discharge was 12.6 J cm^{-2} yr^{-1} (ref. 44). A photochemical source of comparable magnitude has been proposed for both HCN (ref. 45) and H_2CO (ref. 46).

Once produced, HCN would have been hydrolysed and H_2CO would have been destroyed by ultraviolet irradiation, unless they happened to enter a relatively protected microenvironment and managed to combine into more stable compounds. But such combination cannot occur unless the reactants build up to sufficiently high concentrations, which they cannot do unless they are already protected from decomposition. Two possible solutions to this dilemma have been suggested: there may have been a physical mechanism for concentrating the reactants, such as adsorption on a mineral surface[47] or eutectic freezing in a shallow pond or hail particle[48]; alternatively, the condensation reactions may have been catalysed and thus may have operated at much lower concentrations of reactants. An example is glycolonitrile, which is produced readily from HCN and H_2CO and which enhances the rate of HCN oligomerization by one to two orders of magnitude[49].

With HCN and H_2CO thus provided, and with the goal of the RNA world in mind, what came next? Assuming that HCN and H_2CO were present in sufficient quantities to kindle HCN oligomerization, several biologically important molecules would

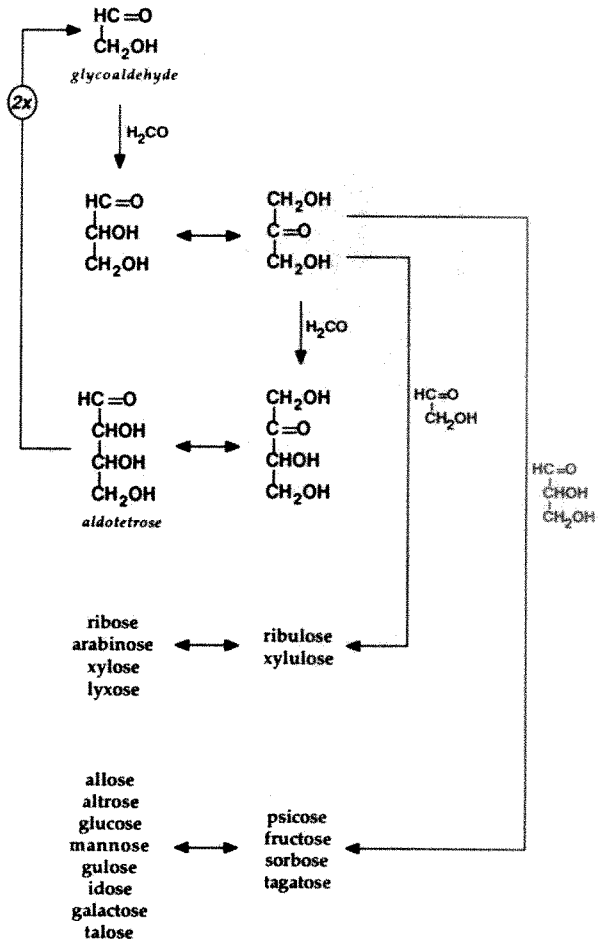
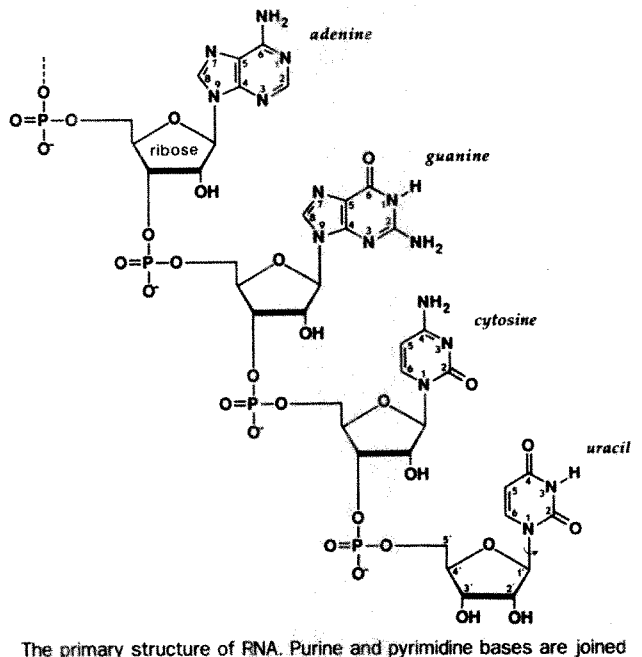


FIG. 2 The formose reaction for the prebiotic synthesis of sugars. Single-headed arrows represent aldol condensations (combination of two aldehydes or ketones to form a β-hydroxyaldehyde or β-hydroxyketone); double-headed arrows represent enolizations (transfer of a proton from an α-carbon to a carbonyl oxygen). The aldotetroses (threose and erythrose) undergo a reverse aldol condensation to yield two molecules of glycoaldehyde. The figure shows D-erythrose as an example of an aldotetrose, but both threose and erythrose can undergo this reaction. Branched-chain and seven-carbon sugars and D- and L-isomers are not shown.

be expected to form (Fig. 1). HCN undergoes a base-catalysed tetramerization reaction to produce diaminomaleonitrile[50], which participates in several interesting chemical reactions, most notably with formamidine to produce 4-aminoimidazole-5-carbonitrile. This leads to the production of adenine, hypoxanthine, guanine, xanthine and diaminopurine[50]. The 4-aminoimidazole-5-carbonitrile intermediate can also be produced directly from diaminomaleonitrile by irradiating with sunlight, so avoiding the use of formamidine which itself must be derived from HCN and NH_3[51].

Diaminomaleonitrile goes on to form higher oligomers of HCN, which appear as a heterogeneous mixture of compounds. Hydrolysis of HCN oligomers yields glycine, alanine, aspartate, diaminosuccinate and smaller amounts of other amino acids[52]. Amino acids have also been synthesized in spark-discharge experiments performed under mildly reducing conditions[53], although the yield and variety of amino acids is substantially lower compared to syntheses performed under strongly reducing conditions. The earliest organisms may have been required to provide their own reducing power, for example using the reduced form of nicotinamide adenine dinucleotide (NAD), NADH, for the biosynthesis of amino acids.

Shifting the focus from HCN to H_2CO, another remarkable polymerization reaction probably took place on the primitive Earth. Accompanying H_2CO would have been smaller amounts of glycoaldehyde, produced either directly or by the condensation of H_2CO in the presence of a catalyst such as calcium carbonate or alumina[54,55]. Glycoaldehyde begins a cascade of aldol condensations and enolizations that rapidly convert most of the available formaldehyde into trioses, tetroses and higher sugars (Fig. 2). This cascade of reactions, collectively termed the formose (or Butlerow) reaction, proceeds autocatalytically because one molecule of threose or erythrose can be cleaved to give two molecules of glycoaldehyde[56]. From a prebiotic perspective, the formose reaction seems a good way to produce ribose and other sugars, although unless they were rapidly used to form nucleosides or some other useful compound, they would have been subject to degradation[57].



The primary structure of RNA. Purine and pyrimidine bases are joined to sugar residues (riboses) to form nucleosides. The purines, adenine and guanine, are attached to ribose by a glycosidic bond between C1′ of ribose and N9 of the base. The pyrimidines, cytosine and uracil, are attached by a bond between C1′ of ribose and N1 of the base. The ribose subunits are joined by a 3′, 5′ phosphodiester linkage to form the ribose–phosphate backbone. Rotation about the glycosidic bond converts the *anti* conformation to the *syn* conformation. Inversion about C1′ would convert the β-conformation to the α-conformation. The numbering schemes for the bases and ribose subunits are shown.

The trouble with RNA

It is a long and difficult road from HCN and H_2CO to the activated nucleoside phosphates that make up RNA (see Box). The self-condensation of HCN to produce purines is such a remarkably simple and efficient reaction that it would be surprising if it did not have some relevance to the early history of life. The prebiotic synthesis of pyrimidines, on the other hand, is more problematic and it is difficult to see how a supply of cytosine or cytosine-containing nucleotides could have been maintained. Cytosine is obtained in about 5% yield when an aqueous solution containing 1.0 M potassium cyanate and 0.1 M cyanoacetylene is incubated at 100 °C for 24 hours[58]. This can hardly be called a prebiotic synthesis because it requires concentrations of reactants that are unlikely to have occurred on the primitive Earth. Cyanoacetylene has been produced by sparking a mixture of CH_4 and N_2 (ref. 58) and presumably could also be obtained from a less reducing mixture. Cyanoacetylene is, however, even more susceptible to hydrolysis than HCN and is unlikely to have built up in significant concentration. There is an alternative route using cyanoacetaldehyde (the first hydrolysis product of cyanoacetylene) and guanidine, although the yields of uracil and, especially, cytosine are low[59]. Furthermore, cytosine would have undergone rapid hydrolysis to uracil (half-life 200 yr at 30 °C)[60]. In contemporary biochemistry, cytidine triphosphate (CTP) is produced by the amination of uridine triphosphate (UTP) in an ATP-dependent reaction.

Another route to the pyrimidines is by acid hydrolysis of HCN oligomers, which can produce 4,5-dihydroxypyrimidine, 5-hydroxyuracil and orotic acid[61]. Orotic acid undergoes a photochemical decarboxylation to give uracil[62], analogous to the enzyme-catalysed decarboxylation that occurs in pyrimidine nucleotide biosynthesis. Uracil can also be produced by condensation of β-alanine and cyanate to give dihydrouracil, followed by photochemical dehydrogenation[63]. Neither of these pathways for the prebiotic synthesis of pyrimidines is especially convincing.

The greatest problem with the pyrimidines is not their synthesis but the difficulty in attaching them to ribose to form pyrimidine nucleosides. First, consider the synthesis of ribose. The formose reaction may offer a good route for the prebiotic synthesis of sugars but it is unclear how ribose could be singled out from the complex mixture of products (Fig. 2). The five-carbon sugars are produced by condensation of glycoaldehyde and glyceraldehyde, accompanied by comparable amounts of tetroses and hexoses produced by the self-condensation of glycoaldehyde and glyceraldehyde, respectively. Even among the pentoses, there are twelve stereoisomers, eight aldoses and four ketoses, to be reckoned with. Perhaps the chemical properties of ribose led to its selective enrichment by some prebiotic fractionation process. The *cis*-(2, 3)-diol structure of ribose and lyxose may have favoured their interaction with a mineral surface where they were relatively protected from hydrolysis compared to arabinose and xylose. The *trans* relationship of the 3-OH and 5-$CH_2OPO_3^{2-}$ groups of ribose-5-phosphate and arabinose-5-phosphate may have rendered them less susceptible to intramolecular cyclization than xylose-5-phosphate and lyxose-5-phosphate[64]. Even if such mechanisms were operating, they would have resulted in a racemic mixture of D- and L-ribose, which still leads to serious difficulties (see below).

Next comes the problem of joining D, L-ribose and a base. When ribose and a purine are heated to dryness in the presence of inorganic salts (Na^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-}), a mixture of α- and β-nucleosides is obtained in about 2–10% yield[65]. A comparable reaction between ribose and pyrimidines is very inefficient (<0.1% yield) and no alternative method for the

synthesis of pyrimidine nucleosides has been found. In biological systems, the pyrimidines are attached to ribose by displacement of a pyrophosphate at the C1 position but attempts to perform a similar displacement under plausible prebiotic conditions have been unsuccessful[66]. Even the successful synthesis of purine nucleosides is plagued by side reactions, including the production of ribosylamine derivatives based on the C6-amine of adenine or the C2-amine of guanine. In biological systems, the purine nucleosides are synthesized by constructing the purine base on a pre-existing ribose-5-phosphate. A similar synthesis under prebiotic conditions seems far more difficult than using the purines formed by HCN polymerization and joining them directly to ribose.

At this point, the best possibility is a mixture of α, β-D, L-purine nucleosides accompanied by a dizzying array of related compounds. Now comes the task of phosphorylating the nucleosides. Heating a mixture of urea, ammonium chloride and hydroxylapatite $[Ca_{10}(PO_4)_6(OH)_2]$ to 100 °C for 24 hours results in a good yield of inorganic polyphosphate[67]. If a nucleoside is added to the mixture, about 20–25% of it is converted to a mixture of nucleoside phosphates, including 2′-, 3′-, 2′,3′-cyclic- and 5′-phosphates, and various 2′(3′),5′-diphosphates[68]. Alternative routes using cyanogen or cyanoacetylene rather than high temperature to drive the reaction have been demonstrated[69], although, as already discussed, these condensing agents were probably not widely available on the primitive Earth.

Finally, the nucleoside phosphates must have been activated to provide a substrate for template-directed polymerization. Adenosine 2′, 3′-cyclic phosphate is in a sense already activated; it can be oligomerized by heating it to dryness[70] or by incubating it at room temperature in the presence of a polyamine[71]. Given the proper stereochemical orientation, the 2′, 3′-cyclic phosphate undergoes condensation with an adjacent nucleoside 5′-hydroxyl to produce a 2′, 5′ or 3′, 5′ phosphodiester linkage[72]. This is comparable to the RNA-catalysed ligation reaction that has been demonstrated using the satellite RNA of tobacco ringspot virus[73] or the genomic RNA of hepatitis delta virus[74].

A more practical approach might be to activate a nucleoside 5′-phosphate and take advantage of the greater nucleophilicity of the *cis*-(2′, 3′)-diol compared to the 5′-hydroxyl for the condensation between a 5′-phosphate and 2′(3′)-hydroxyl. Adenosine 5′-phosphate can be converted to the diphosphate and subsequently the triphosphate by incubating it with carbamyl phosphate in the presence of a divalent metal cation[75]. Carbamyl phosphate is itself produced by condensation of cyanate and inorganic phosphate in aqueous solution[76]. Nucleoside 5′-polyphosphates have also been obtained by heating a solution of nucleotides and inorganic polyphosphate to dryness[77].

Could this long and difficult road to activated mononucleotides be travelled without biological catalysts (or an organic chemist) to lead the way? Suppose that somehow it did happen; that given a few hundred million years and an entire globe of microenvironments, there was a special time and place in which a rich soup of activated mononucleotides formed. The only flaw in this hypothetical garden of Eden is that the soup would contain a racemic mixture of D- and L-nucleotides, a necessary consequence of the fact that the prebiotic environment was achiral, which, it turns out, makes RNA replication very difficult. By a strange twist of nucleotide chemistry, D-mononucleotides in the *anti* conformation and L-mononucleotides in the *syn* conformation tend to form very close structural homologues when bound to a complementary template[78] (Fig. 3). As a result, the template-directed synthesis of one enantiomer is strongly inhibited by the presence of the other[79]. The 'wrong' enantiomer adds to the 3′ end of a growing chain, which distorts the 3′ terminus and thus prevents further chain elongation.

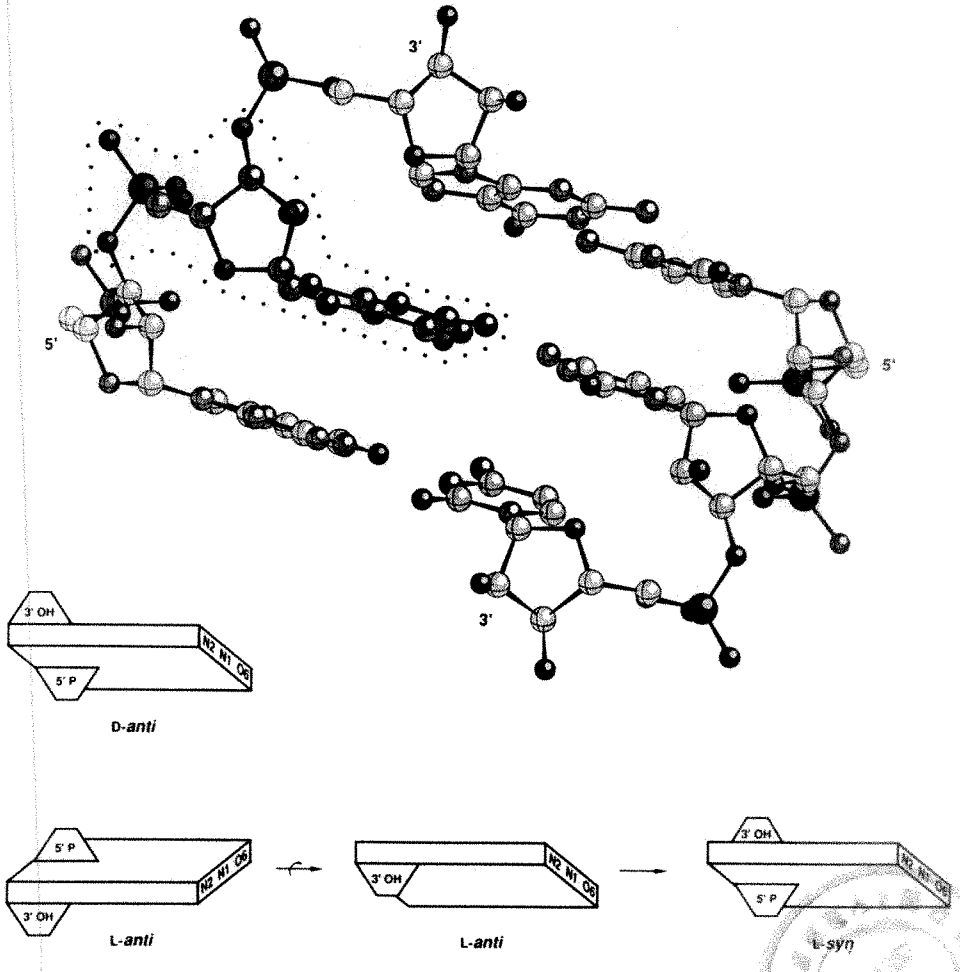


FIG. 3 Top, three successive guanosine · cytosine pairs of the A-form of RNA projected onto a plane perpendicular to the diad axis. The middle guanosine residue is enclosed in a dotted box. Atom coordinates are based on the crystal structure of A-RNA[96]. Graphical representation was performed using *Ball & Stick* software on a Macintosh computer. Bottom, the close structural homology between D-*anti*- and L-*syn*-guanosine mononucleotides when bound to a complementary template. The D-*anti* form corresponds to the middle guanosine residue shown above. Imagine reflecting this in a mirror to produce the L-*anti* form, which is rotated about its long axis to orient the base for complementary pairing and finally rotated about the glycosidic bond to produce the L-*syn* form.

If one admits both α- and β-nucleotides to the hypothetical garden of Eden, another cross-inhibition phenomenon is likely to occur. α-D-mononucleotides in the *syn* conformation and β-D-mononucleotides in the *anti* conformation also form close structural homologues[80] and any combination of α-D-*syn*-, α-L-*anti*, β-D-*anti*-, or β-L-*syn*-mononucleotides is likely to be a problem. The addition of arabinose, other pentoses and the hexoses is an open invitation to template-directed chaos. It is difficult to imagine how a genetic message could be transferred from template to complementary product in the face of all this isomeric interference. The most reasonable interpretation is that life did not start with RNA. The RNA world came into existence after many of the problems associated with the prebiotic synthesis and template-directed replication of RNA had been solved. This implies that there was a simpler genetic system, or systems, that preceded RNA and that evolutionary advances made by the ancestral system were somehow carried over to the RNA world.

The origin of self-replication

We know very little about the chemical nature of the first self-replicating molecule. There are four general requirements for the primordial genetic material, regardless of its chemical nature. First, it must have had informational properties, suggesting that it was some type of heteropolymer, composed of at least two monomeric subunits. Second, it must have been capable of directing the ordered assembly of monomeric starting materials to form additional copies of itself. Third, the monomeric starting materials must have been readily available, at least in some locale on the primitive Earth. Fourth, the genetic material must have been sufficiently stable for its rate of reproduction to exceed its rate of decomposition. If the goal is the eventual origin of an RNA world, then a requirement for an evolutionary pathway from the first self-replicating molecule to a genetic system based on RNA must be added.

There are three ways in which the difficulties in arriving at an RNA world may have been reduced. One is that before the origin of self-replication, there was a period of chemical evolution[81], during which non-instructed processes led to progressive alteration of the chemical composition of the environment. The prebiotic environment can be thought of as a flow reactor, driven by the supply of HCN, H_2CO and other high-energy starting materials, and characterized by a steady-state distribution of reactants and products. In this context, the formose reaction for instance, may not have been quite so unruly; rather than the cascade of all possible 3-, 4-, 5-, 6- and 7-carbon sugars obtained from batch polymerization of H_2CO, there might have been more limited distribution of sugars determined by the differential rates of formation and decomposition of the various compounds[82]. Non-instructed chemical ordering may have led to very complex structures, such as colloidal aggregates of polypeptides[18] and even self-propagating membranous vesicles[83]. These complex structures may have had a profound influence on the local environment and may have set the stage for the emergence of a self-replicating system.

A second possibility is the intervention of a primitive self-replicating system chemically unrelated to RNA, which may have helped to bias the environment in a way that made the appearance of RNA or RNA-like molecules more likely. The most carefully constructed hypothesis along these lines concerns the possibility of self-replicating clay minerals[13], where the genetic material is considered to be a distribution of ionic charges, embedded organic compounds or structural defects along a mineral surface that serves as a template for the formation of additional mineral layers. At one extreme, these clay 'organisms' may have come and gone, leaving their mark by altering the local environment. At the other extreme, they may have developed the capacity to synthesize RNA or RNA-like molecules that were used initially as catalysts or cofactors in the clay world but eventually themselves became self-replicating and took on the role of genetic material[84].

The third advance on the way to an RNA world may have been the appearance of a self-replicating chemical system similar to RNA but simpler in the sense that it demanded less from the chemistry of the prebiotic environment[78,85]. For example, the difficulties associated with the prebiotic synthesis of pyrimidines could be circumvented if the genetic material were based on purines alone. Non-standard pairing combinations such as adenine: inosine, adenine: xanthine and guanine: isoguanine have been suggested[8,86], although there is no experimental evidence that they are suitable for template-directed polymerization. The backbone might have been simpler, with ribose replaced by something that was not accompanied by dozens of closely related compounds or subject to enantiomeric cross-inhibition. A number of flexible, acylic nucleoside analogues that fit this description have been proposed[78,87] (Fig. 4).

The compound that has been studied in the greatest detail is the glycerol-derived nucleoside analogue (compound *b* in Fig. 4). In principle, it could be synthesized by condensation of H_2CO and glycerol followed by addition of a base. This should produce a mixture of various mono- and di-substituted glycerol derivatives but this would be far simpler than the assortment of ribosyl- and other sugar derivatives that would accompany a nucleoside. This compound, either non-phosphorylated or phosphorylated at both hydroxyl positions, is an achiral molecule. It becomes chiral when incorporated into a heteropolymeric structure. The glycerol-derived analogue of guanosine bisphosphate, when activated at both phosphates, undergoes a poly(C)-directed oligomerization reaction to form pyrophosphate-linked products[88]. A pyrophosphate-linked oligomer formed from deoxycytidine bisphosphate has also been used as a template to direct the oligomerization of deoxyguanosine bisphosphate derivatives (A. W. Schwartz, personal communication). Thus there could have been a genetic molecule with a glycerol-phosphate backbone that replicated by a chemical process and was able to undergo darwinian evolution.

Chemical self-replication, in the glycerol-phosphate system or in any RNA-like system, faces several obstacles. One of the most serious is the need for end-to-end copying of the template before the development of a mechanism to ensure that replica-

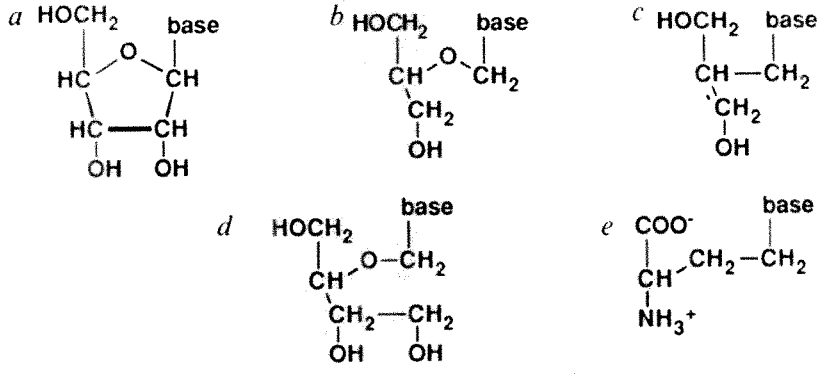


FIG. 4 Comparative structure of a nucleoside and of four simple acyclic nucleoside analogues. *a*, nucleoside; *b*, glycerol-derived acyclonucleoside; *c*, acrolein-derived nucleoside analogue; *d*, erythritol-derived acyclonucleoside; *e*, aspartate-derived nucleoside analogue.

tion begins at a discrete initiation site. In the RNA world this problem may have been solved by a 'genomic-tag' structure that directed a primitive replicase to the appropriate initiation site[89]. In the early stages of self-replication, a chemical tag could have demarcated one end of the template or a chemical ligation mechanism could have joined partial copies together to form complete products.

Another important obstacle to chemical self-replication, which has been demonstrated in experimental systems, is the tendency of molecules with regions of self-complementarity to form intramolecular duplex structures[90]. It is difficult to envisage a solution to this problem in the absence of a factor such as single-strand binding proteins, because the conditions must favour short duplex regions so that template-directed synthesis can occur yet disfavour long duplex regions that would produce stable secondary structures. There may be alternative helical backbones that have non-cooperative properties so that longer duplex structures are increasingly disfavoured[78]. In any event, the development of an unwinding activity would have been a top priority during early evolutionary history.

Finally, there is the problem of accurate copying of the genetic information in the absence of a sophisticated replicase enzyme. The selectivity of complementary base pairing provides the chemical basis for information transfer, with the fidelity depending on the particular bases involved (highest for cytosine→guanine, lowest for adenine→uracil)[91]. The efficiency of copying also depends on base sequence, with selection pressure favouring those sequences that can be copied most readily. One of the tasks of a polymerase enzyme is to 'smooth' these differences so that a broader range of sequences can be explored[92]. But given the limits on genome size (as determined by copying fidelity[29]) and on sequence heterogeneity, would there have been enough potential sequences remaining to evolve a smoothing function that could further expand the range of potential sequences? A crude replicase activity must have arisen so that copying fidelity could be improved, genome size could be increased and ever more sophisticated catalysts could be developed.

Transition to an RNA world

Let us suppose that after a period of chemical evolution, with or without further augmentation by a genetic system completely unrelated to RNA, a genetic system arose based on some simple RNA-like molecule. It is not inconceivable that a genetic system completely unrelated to RNA would have invented RNA for its own purposes. With so many design problems associated with RNA synthesis, it seems more likely that a simple RNA-like molecule was invented instead. Once a genetic system based on an RNA-like molecule existed, the domain of nucleotide chemistry would have been more accessible and the invention of RNA would have been more practical. Why should an increasingly sophisticated RNA-like world come to synthesize mononucleotides rather than optimize the synthesis of its own monomeric units? The answer must be that mononucleotides somehow conferred a selective advantage to the RNA-like world, either by improving its information storage properties or by enhancing its catalytic abilities.

Reviewing the catalytic properties of known RNA enzymes[93], RNA catalysis seems to be limited to sequence-specific cleavage/ligation reactions involving nucleic acid substrates. But the chemical properties of the various functional groups within RNA suggest a broader range of catalytic function (Table 2). Of particular importance to RNA, but not to any of its likely predecessors, is the ribose 2′ hydroxyl. A terminal *cis*-(2′, 3′)-diol is a good nucleophile and an internal 2′-hydroxyl can function either as a nucleophile[22] or as a determinant of RNA tertiary structure[94,95]. Because of the 2′-hydroxyl, RNA may have enhanced the structural and catalytic properties of the RNA-like world and its production could have been a favourable event.

We do not know how the transition from an RNA-like to an RNA world was made. There are several ways in which RNA may have gained entry. One is that nucleotides or nucleotide derivatives were generated to perform some useful function in the RNA-like world and then took root as cofactors in primitive metabolic processes. The RNA cofactors progressed from monomeric to oligomeric forms and eventually became autonomously self-replicating. Another possibility is that mononucleotides were at first inadvertently incorporated into the ancestral genetic material but this proved selectively advantageous. It is not clear how random misincorporations could have been useful or how the incorporation of mononucleotides could have been limited to specific sites. Somehow the genetic material took on more and more of the character of RNA without forsaking its past evolutionary accomplishments. A third possibility is that mononucleotides, originally present as cofactors, became oligomerized in a template-dependent way to give products that contained only RNA components. The ancestral genetic material directed both its own replication and the 'transcription' of RNA. RNA became autonomously self-replicating and either shed its RNA-like predecessor or, through reverse transcription, replaced the predecessor with DNA.

The transition to an RNA world, like the origins of life in general, is fraught with uncertainty and is plagued by a lack of relevant experimental data. Researchers into the origins of life have grown accustomed to the level of frustration in these problems. Perhaps it is time for a new group of scientists to join in the frustration. The discovery of catalytic RNA and the appreciation of the dual role of RNA as both genetic material and catalyst have kindled new interest in the evolution of RNA and the origins of life. It is time to go beyond talking about an RNA world and begin to put the evolution of RNA in the context of the chemistry that came before it and the biology that followed. □

TABLE 2 Catalytic potential of RNA

Component	Metal-ion coordination	General acid catalysis	General base catalysis	Nucleophile
Ribose	2′OH			2′OH, 2′(3′)-diol
Phosphate	O^-	OH	O^-	
Adenine	N7		N1	
Guanine	N7	N1	N7	
Cytosine	N3		N3	
Uracil	O2	N3		

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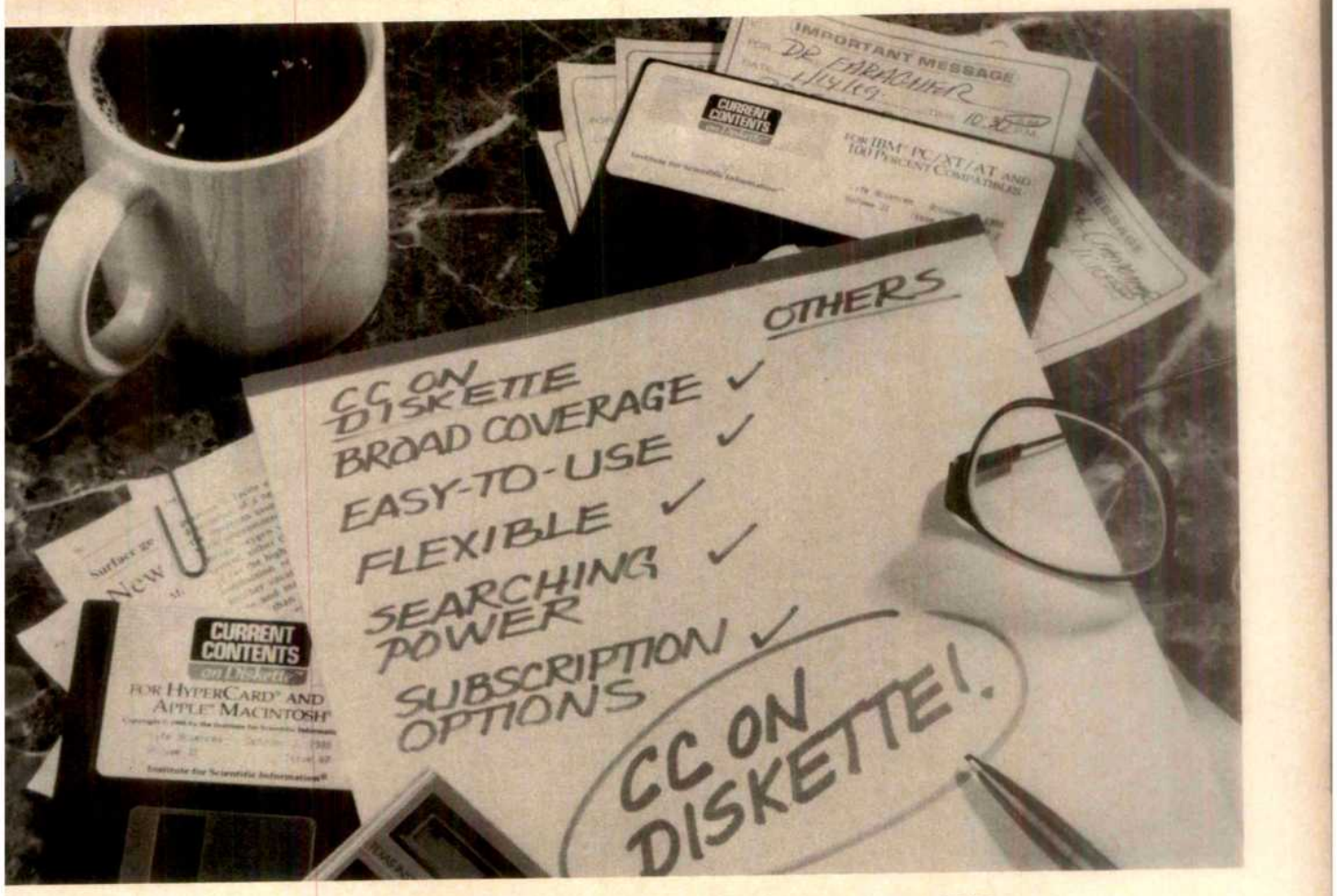
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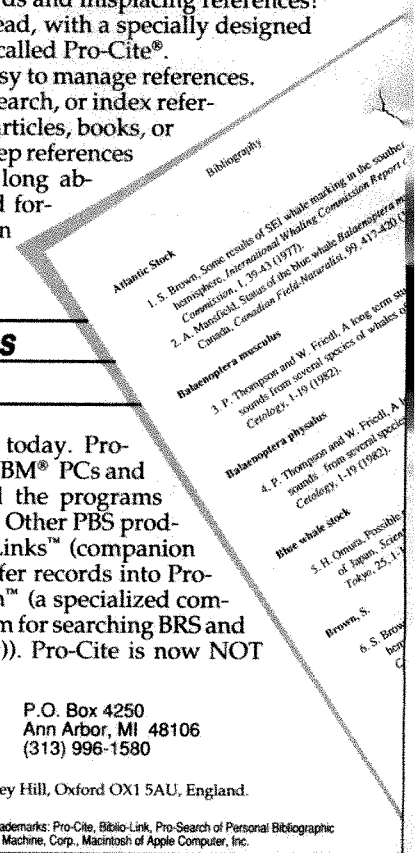
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Structure of tumour necrosis factor

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Tumour necrosis factor is a trimeric molecule, each subunit of which consists of an antiparallel β-sandwich. Individual subunits form the trimer by a novel edge-to-face packing of β-sheets. A comparison of the subunit fold with that of other proteins reveals a remarkable similarity to the 'jelly-roll' structural motif characteristic of viral coat proteins.

TUMOUR necrosis factor (TNF) plays a key role as a polypeptide mediator of inflammation and the cellular immune response. It is secreted by macrophages and is a member of the class of molecules termed cytokines, a group which includes the interferons and interleukins. These hormones form a complex network of interactive signals that regulate their own production and the growth and differentiation of cells involved in inflammation, immunity and haemopoiesis[1]. TNF was first characterized by its *in vivo* antitumour properties and *in vitro* cytotoxicity to certain transformed cell lines[2]. Lipopolysaccharide (LPS) is a potent trigger for TNF production[3,4] and the effects of LPS such as fever and shock seem to be mediated by TNF. TNF also displays antiviral activity[5,6] and plays a protective role in malarial infections[7]. Conversely, it is implicated in the pathogenesis of cachexia (wasting)[4], rheumatoid arthritis and inflammatory tissue destruction[8,9].

TNF is secreted as an unglycosylated 157-amino-acid polypeptide of relative molecular mass (M_r) 17,350 (17 K). Its primary structure is evolutionarily conserved[10], and in its active form the protein is thought to be trimeric[11]. TNF binds to a receptor, believed to be a glycoprotein of $M_r \sim 75$ K[11-17].

We report here the determination of the structure of the TNF trimer at 2.9 Å resolution by X-ray crystallography. The main-chain fold of a TNF subunit shows a remarkable similarity to the 'jelly-roll' structural motif of viral capsid proteins, making it unique among known non-viral proteins.

Structure determination

Material of high purity was obtained from the recombinant human TNF gene expressed in *Escherichia coli* as the 17 K protein. Crystals of TNF grew by vapour diffusion as hexagonal rods ($0.5 \times 0.5 \times 1.5$ mm) from ammonium sulphate solution at room temperature (N.P.C.W. and S. Marcinowski, unpublished observations). The crystals belong to the trigonal space group $P3_121$ with unit cell dimensions $a = b = 166$ Å, $c = 93$ Å and diffract to 2.9 Å resolution. Crystal density measurements using the Ficoll density gradient method indicated that the asymmetric unit probably contained two trimers; the crystal solvent content was therefore 65%.

Data were generated and collected using synchrotron radiation at the SERC Daresbury Laboratory and a Xentronics multiwire area detector at Oxford (Table 1). The presence of six subunits of unknown orientation within the asymmetric unit made the solution of heavy atom derivatives problematic; self-rotation function calculations failed to locate the non-crystallographic 3-fold axes of the two trimers. Analysis of difference Patterson maps for putative heavy-atom derivatives was undertaken using programs (GROPAT) written specifically for this project (E.Y.J. and D.I.S., manuscript in preparation). This analysis provided the heavy atom positions for an $Hg(CH_3CO_2)_2$ derivative that had one heavy-atom site per subunit. Difference Fouriers then revealed two other usable multiple site derivatives of poorer quality. Phasing statistics are given in Table 1.

Solvent flattening was applied to the three 6 Å SIR maps for these derivatives, in turn, and a 'best' map was then produced as a weighted average of these. From this starting point, gradual and very careful phase extension to 4 Å was carried out using phase information from the first derivative combined with solvent flattening. Beyond 4 Å no phase information was available from the heavy-atom derivatives. Further phase extension was achieved by solvent flattening and averaging using the Bricogne algorithms[19]. We performed 3-fold averaging separately about the two trimers. After initial cycling at 4 Å, very gradual phase extension to the limit of the native data, 2.9 Å, was carried out. Although the positions of both non-crystallographic 3-fold axes refined satisfactorily, one of the two trimers (the second) showed significantly poorer averaging statistics. After cycling to convergence at 2.8 Å, it was decided to remove the constraint of 3-fold averaging for the second trimer and continue with this modified cycle of partial averaging and solvent flattening. Surprisingly, this gave electron density of an improved quality for both trimers. Averaging and phase extension statistics are given in Table 1.

An unambiguous chain trace was quickly established for one of the three subunits of trimer one, and confirmed by fitting the amino-acid sequence. Apart from two regions of high mobility there are no main-chain breaks, side-chain density is well defined, and a high percentage of main-chain carbonyl bulges are visible. A representative portion of the map is shown in Fig. 1. The final model comprises 153 residues and is complete but for the first 4 N-terminal amino acids for which clear electron density is not visible. The structure has now been refined using X-PLOR[20] on a CONVEX C210. The R-factor is at present 22.2% (on all data from 6 to 2.9 Å) after restrained B-factor refinement with excellent stereochemistry (r.m.s. deviation from

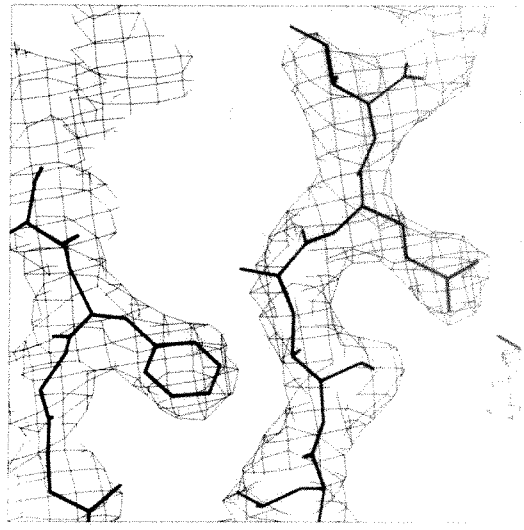


FIG. 1 Portion of the electron-density map (before refinement) viewed edge-on to the β-sandwich. Phe 152 in β-strand I and Glu 135 in β-strand H are shown in the centre of the figure. Electron density contoured at an arbitrary level is represented by chicken wire.

ideal bond lengths of 0.013 Å) and no model for the solvent structure.

Description of the structure

TNF is a compact trimer composed of 3 identical subunits of 157 amino acids (Fig. 2). The overall shape of the monomeric subunit is wedge-like with length 50 Å and maximum breadth 30 Å. It is essentially a β-sandwich structure formed by two antiparallel β-pleated sheets and has the topology of the classic viral jelly-roll motif (Fig. 3*a*). The upper sheet (sheet 1) is kinked with three long strands supplemented for half the sheet by a loop of two additional strands. The lower sheet (sheet 2) comprises five strands of steadily decreasing length. The middle strand of this sheet consists of the last nine C-terminal residues; the C terminus is thus firmly locked into position. By contrast, the flexible N-terminal region runs past a neighbouring subunit in the trimer before the chain is incorporated into the protein core as a β-strand in sheet 2. It has previously been concluded[4] that the complete carboxy-terminal sequence is essential for bioactivity, whereas amino-terminal deletions of up to eight residues may be sustained without loss of bioactivity. This is consistent with the maintenance of structural integrity in sheet 2. The single disulphide bridge (residues 69 and 101) stretches between the two longest intersheet loops. This region (in particular residues 102–113 in the larger of these loops) comprises the other area of high main-chain mobility.

The subunits associate tightly about the 3-fold to form a compact conical-shaped trimer of length 50 Å and maximum breadth 45 Å. The interaction is through a simple edge-to-face packing of the β-sandwich (Fig. 2*b*). The edge of the β-sandwich consisting of strands F and G from one subunit lies across sheet 2 of a 3-fold related subunit. The carboxy terminus lies close to the 3-fold axis. The subunit β-sandwich has the expected filling of tightly intercalating large apolar residues. The outer surface of the trimer, mainly comprising the outerside of sheet 1, is decorated with charged and polar side-chains. The remaining face, that of sheet 2 which mediates the 3-fold edge-to-face interaction, is predominantly polar in character.

Structural homology

The connectivity for the standard eight strands of the viral jelly-roll motif and the equivalent elements of secondary structure in the TNF subunit is identical. A series of comparisons between TNF and other β-barrel proteins has been carried

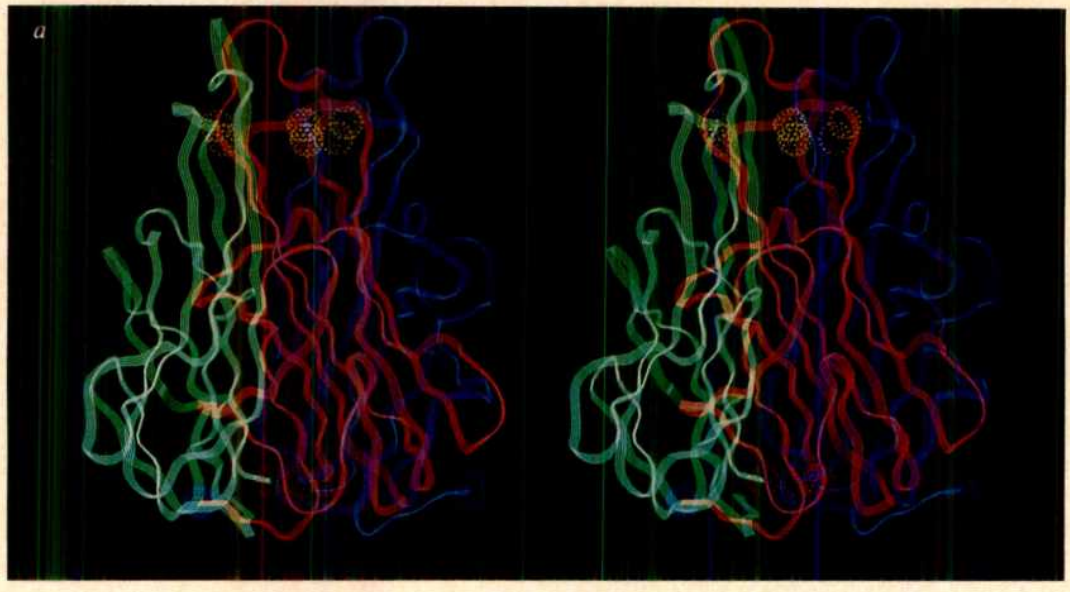




FIG. 2 *a*, Ribbon representation of the trimer with the 3-fold axis vertical. Individual subunits are colour-coded blue, green and red. N termini are marked by blue spheres and C termini by red. Yellow spheres near the top of the molecule indicate the disulphide bridges. *b*, Edge-to-face packing of the monomers in the TNF trimer. The view is down the 3-fold axis showing a narrow slab of the trimer with β-strands running into and out of the page.

out using SHP[21], a program which allows automatic definition of the 'equivalent' residues based on superposition of C_α positions.

Foot and mouth disease virus (FMDV) provides an example of the degree of structural homology found between the capsid proteins VP1, VP2 and VP3 of a picornavirus[22]. Typically 60–70% of the residues are matched with r.m.s. deviations of up to 3.2 Å. The immunoglobulin domain is also an antiparallel β-sandwich but with a totally different connectivity; a comparison of this motif with the jelly-roll fold of FMDV VP3 yields significantly lower values with only about 30% of the VP3 residues matched with r.m.s. deviations of 3.7–4.0 Å. These two results provide useful benchmarks when considering the data for TNF. For a non-jelly-roll β-barrel structure the number of equivalenced TNF residues varies from about 40 to 50% with r.m.s. deviations of between 3.5–4.0 Å. Concanavalin A[23] is an intermediate structure, a considerable portion of this more elaborate molecule can be matched to the viral fold[24]. This is reflected in the comparison with TNF, for which 64% of the residues of TNF can be matched but with a large r.m.s. deviation of 4.04 Å. The number of equivalenced TNF residues is consistently in excess of 60% with r.m.s. values frequently below 3.5 Å when matched against structures with the jelly-roll motif. Several such comparisons are particularly noteworthy. The structural similarity between TNF and the jelly-roll domain of influenza haemagglutinin (HA)[25] is as close as that between HA and FMDV VP3. In the case of two viral capsids, tomato bushy stunt virus (TBSV)[26] and adenovirus hexon[27], a pair of jelly-roll structures have been found on the same polypeptide chain, presumably as a result of gene duplication. In both cases, TNF is at least as similar to one jelly roll as the two jelly rolls are to each other. The most striking similarity, however, occurs between TNF and satellite tobacco necrosis virus (STNV),[28] a very simple $T = 1$ plant virus. 71% of TNF residues can be equivalenced to residues in STNV with only 3.3 Å r.m.s. deviation (Fig. 3*b*). Thus TNF is structurally as similar to STNV as this protein is to other viral coat proteins.

The structural similarity between the capsid proteins of the small spherical plant viruses, and subsequently the picornaviruses, has been taken to indicate a common evolutionary precursor[29,30]. The structural similarity described here points to the divergent evolution of TNF and the viral proteins from a common ancestral jelly-roll protein. The identification of additional common characters adds weight to this hypothesis. First, the solvent-accessible outside face in the TNF trimer and in the viral capsid are equivalent. Second, the packing of jelly rolls in the capsid of adenovirus is reminiscent of the TNF trimer. The adenovirus hexon[27] contains two jelly-roll domains (P1 and P2) which pack about a quasi 6-fold axis in the hexon. The adenovirus capsid is constructed out of these hexagonal plates such that copies of P1 or P2 from three plates lock into a 3-fold arrangement about each corner of the hexon. Unlike the case in picornaviruses, the longest axis of the β-barrel is roughly normal to the adenovirus surface; hence these 3-fold clusters of P1 or P2 and the TNF trimer have a common general mode of packing. The versatility of the basic jelly-roll motif in fulfilling

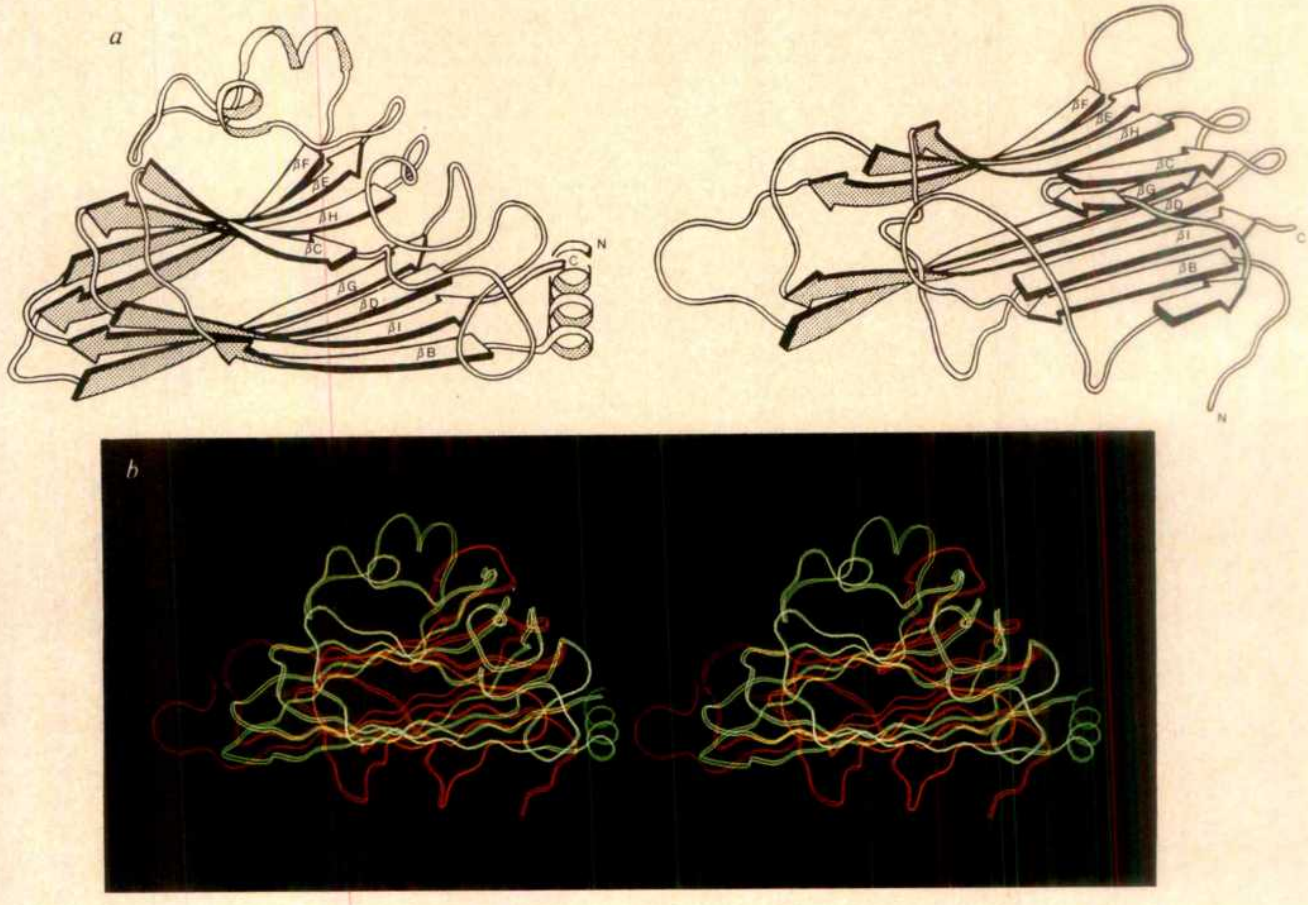


FIG. 3 *a*, Diagrammatic sketch of the polypeptide fold for STNV (top) and TNF (bottom). The β-strands are shown as thick arrows in the amino-to-carboxy direction and are labelled according to the viral convention. An insertion in TNF results in severe truncation of the N-terminal half of β-strand C and addition of short, extra β-strands to the outer edges of both upper and lower sheets. The TNF trimer 3-fold axis would be horizontal in this orientation. *b*, Superposition of ribbon representations for STNV (green) and TNF (red). The upper β-sheet forms the solvent-accessible surface in both cases.

TABLE 1 Structure determination statistics

Data set	Resolution (Å)	Reflections unique (% complete)	Data collection and phasing: *R* merge‡ (all data)	Average isomorphous difference	FM (all data, 20–6 Å)	⟨FHA⟩/⟨ETOT⟩ (for minimum FM cutoff)	Wang *R*-factor 6 Å	4 Å
Native (film)*	2.9	24,385 (76%)	0.125	—				
Native (film)	3.4	16,031 (78%)	0.075					
Native (XEN)	5.0	5,582 (85%)	0.056					
$Hg(CH_3CO_2)_2$ (XEN: 5 Å; 3.5 Å)	3.5	14,779 (80%)	0.073	19.11%	0.40	1.7 (0.0)	0.24	0.20
†$K_2Pt(CN)_4$ (XEN, film)	3.4	11,944 (58%)	0.080	17.43%	0.34	2.2 (0.5)	0.26	
K_2HgI_4 (XEN)	5.0	5,977 (91%)	0.095	27.46%	0.34	2.4 (0.5)	0.25	

	Averaging: Averaging *R*-factor	Correlation coefficient	Real space *R*-factor: TR1	TR2
TR1 and TR2 averaging (2.9 Å)	0.28	0.77	0.27	0.43
TR1 averaging (2.8 Å)	0.19	0.89	0.26	—

Film data were collected on 2° oscillation photographs (3.4 Å resolution) or 1° oscillation photographs (2.9 Å resolution), processed and scaled using standard programs[21]. The data sets collected on the Xentronics area detector (XEN) were taken as 0.2° oscillation frames and processed using the XENGEN program package (A. Howard, XENGEN manual). About 20 heavy-atom compounds were screened for isomorphous derivatives, complete data sets were collected for 15 of these, of which 3 yielded usable data. FHA, heavy-atom structure factor; ETOT, residual lack of closure; FM, figure of merit. Phasing statistics were obtained before any phase modification by solvent flattening. Wang *R*-factor, the crystallographic *R*-factor between observed structure factor amplitudes and those calculated from the solvent flattened map ($R = \sum |(|F_o| - |F_c|)|/\sum |F_o|$). Averaging *R*-factor, the crystallographic *R*-factor between observed structure factor amplitudes and structure factor amplitudes calculated from the averaged and solvent flattened map. Real space *R*-factor, the *R*-factor between non-crystallographically related electron density. Correlation coefficient, $C = \sum (\langle F_h \rangle - |F_o|)(\langle F_h \rangle - |F_c|)/[\sum (\langle F_h \rangle - |F_o|)^2 (\langle F_h \rangle - |F_c|)^2]^{1/2}$, where $|F_o|$ and $|F_c|$ are observed and calculated structure factor amplitudes placed on the same relative scale for each resolution shell, and $\langle F_h \rangle$ is the mean observed amplitude in that shell. TR1, first trimer. TR2, second trimer.

* Including partials. † 10% contribution from Enraf Nonius FAST data. ‡ $R\,\text{merge} = \sum_j \sum_h |I_{h,j} - \langle I_h \rangle| / \sum_j \sum_h \langle I_h \rangle$.

very different packing criteria has been commented on previously[24,27].

Conclusions

The structures of three cytokines have so far been established: interleukin-2 (ref. 31), interleukin-1β (ref. 32), and now TNF. Each has a different structural motif. Thus the overlapping biological roles of these molecules are not reflected in their overall structures.

TNF is the first non-viral protein to contain the classic jelly-roll motif. It is a compact molecule shorn of most of the insertions which decorate the jelly roll in the typical viral protein. The basic antiparallel β-sandwich of the jelly roll provides a very stable core arrangement of secondary structure from which connecting loops can provide a myriad of structural variations, a similar principle to that used by the topologically distinct immunoglobulin superfamily. One reason for TNF's rather austere jelly roll could be that it has no need of great variability as it is not seeking to evade host surveillance. Similarly the STNV coat protein is free from the pressures of escaping an immune system and the more demanding packing requirements associated with the conformational switching of more complex viruses. Hence the greatest similarity occurs between TNF and the coat protein of the simplest of the spherical plant viruses.

If one accepts the case for divergent evolution then our observation accords with the hypothesis of Rossmann *et al.*[30] that the viral jelly roll originated from a non-viral receptor binding protein. This homology may in the future provide insight into the functional relationships of these diverse proteins. Our present knowledge suggests that a crucial event in the origins of at least a major class of viruses was the acquisition of the ability to assemble a stable protein shell from subunits based on the jelly-roll motif. It may well be that with the structure of TNF we can start to piece together these origins. □

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A laminin-like adhesive protein concentrated in the synaptic cleft of the neuromuscular junction

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A striking example of topographic specificity in synapse formation is the preferential reinnervation of original synaptic sites on denervated muscle fibres by regenerating motor axons. This specificity is mediated by the basal lamina of the synaptic cleft. A glycoprotein, s-laminin, has now been identified that is selectively associated with synaptic basal lamina and is recognized by motoneurons. Molecular cloning reveals that s-laminin is a novel homologue of laminin, a potent promoter of neurite outgrowth.

IN most vertebrate skeletal muscles, each muscle fibre is innervated at a single site by a single motor axon. Injuring the motor nerve leaves the muscle denervated, but axons can regenerate to form new neuromuscular junctions that resemble their normal counterparts in structure and function. A striking and consistent feature of reinnervation is that it is topographically specific: axons preferentially form new junctions at original synaptic sites[1-3]. The fidelity of this process—in some cases more than 95% of the regenerated synapses form on the ~0.1% of the muscle fibre surface that was originally synaptic[3]—indicates that there are factors closely associated with original synaptic sites that axons recognize.

We have shown previously that some of these factors are associated with the basal lamina (BL) that ensheathes each muscle fibre and passes through the synaptic cleft at the neuromuscular junction[3,4]. Subsequently, we[5,6] and others[7,8] generated antibodies that selectively stained synaptic sites on muscle fibre BL, revealing molecular correlates of the functional specialization demonstrable during reinnervation. Here, we identify one such synaptic antigen as a novel homologue of laminin, a glycoprotein of the BL already known to be adhesive for neurons[9]. Because the distribution of this laminin-like protein is restricted to a subset of BLs, including synaptic BL, we call it s-laminin. Its location, bioactivity and resemblance to laminin suggest that s-laminin is involved in the formation or stabilization of synapses.

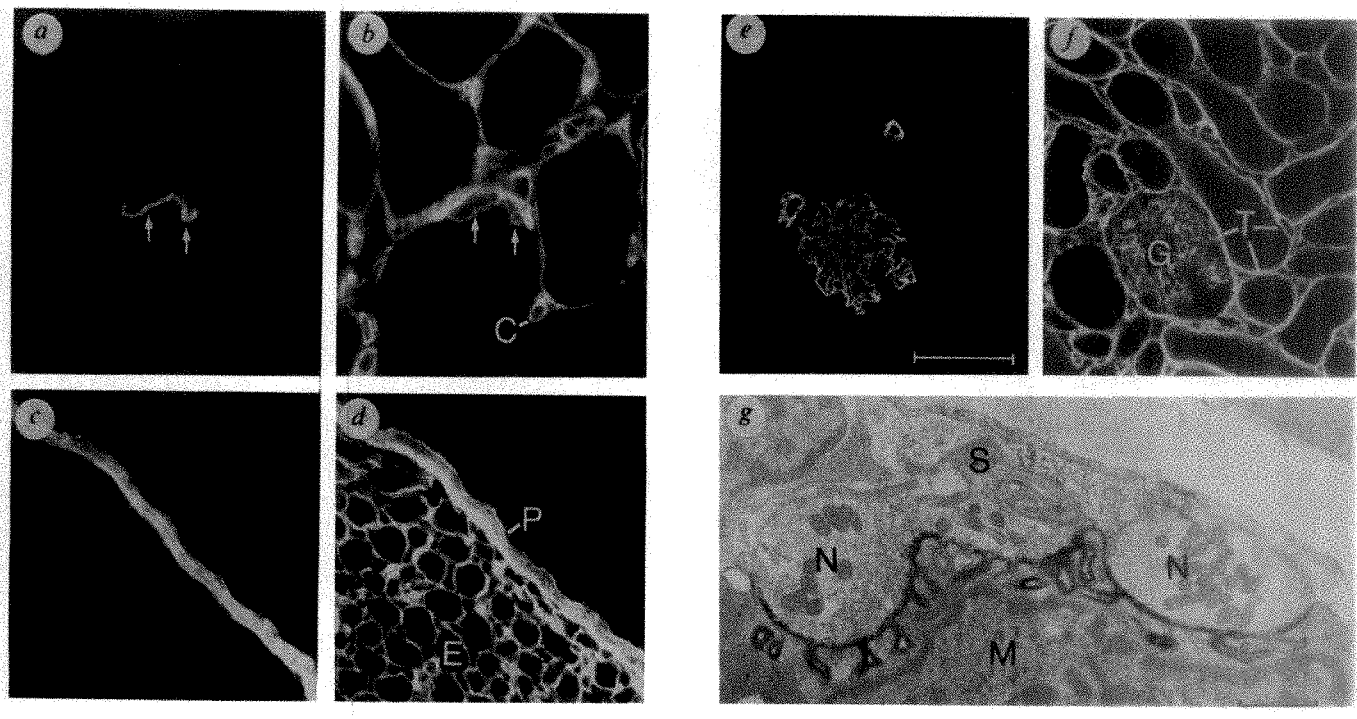


FIG. 1 S-laminin is restricted to a subset of laminin-rich BLs. Cryostat sections of rat skeletal muscle (*a, b*), sciatic nerve (*c, d*) or kidney (*e, f*) were doubly stained with anti-s-laminin mAb D7 plus fluorescein goat anti-mouse IgG (*a, c, e*) and with anti-laminin serum plus rhodamine goat anti-rabbit IgG (*b, d, f*), as described[44]. S-laminin is concentrated in synaptic (arrows) but not extrasynaptic or capillary (C) BL in muscle (*a, b*), in perineurial (P) but not endoneurial (E) BL in nerve (*c, d*) and in glomerular (G) but not tubular (T) BL in kidney (*e, f*). *g*, Electron micrograph of a neuromuscular junction from a muscle that had been stained with mAb C1 plus horseradish peroxidase second antibody[44]. Reaction product is concentrated in synaptic BL between nerve terminals (N) and muscle fibre (M); extrasynaptic and Schwann cell (S) BLs are not detectably stained. In experiments not shown here, we used methods described in ref. 5 to show that mAbs to s-laminin stained the BL in the synaptic cleft, rather than the pre- or postsynaptic membranes. Scale bar in *e*, 50 μm for *a–d* and 100 μm for *e, f*. Scale bar in *g*, 1 μm.

Restricted distribution of s-laminin

S-laminin was initially defined by an antiserum (JS-1) to an extract of lens capsule BL, which stained synaptic BL[5]. Because the antiserum was not monospecific, we generated four monoclonal antibodies (mAbs; C1, C4, D5 and D7; see refs 6, 10) that recognized JS-1-related antigens: each stained synaptic BL (Fig. 1*a*, *g*) and staining by each was blocked by preincubation of the sections with JS-1 (data not shown). All four mAbs recognized the same polypeptide, s-laminin (see below), but they differed in species cross-reactivity and in reactivity with denatured antigen, indicating that each mAb recognizes a distinct epitope. The selective staining of synaptic BL by these mAbs is therefore likely to reflect restricted distribution of s-laminin rather than differential accessibility of a uniformly distributed determinant.

Although s-laminin is concentrated at synaptic sites in muscle, it is abundant in some non-muscle BLs. In peripheral nerve, all four mAbs to s-laminin stained perineurial BL more intensely than endoneurial BL (Fig. 1*c*). In kidney, these mAbs stained glomerular BL more intensely than tubular BL (Fig. 1*e*). Within muscle, arterial BL was stained more strongly for s-laminin than venous or capillary BL. All BLs were strongly and similarly stained by antibodies to laminin (Fig. 1*b*, *d*, *f*) and to a heparan sulphate proteoglycan[11]. Thus, s-laminin is restricted to a subset of BLs both in muscle and elsewhere. Interestingly, in the tissues examined so far, BLs rich in s-laminin are apposed on both sides by cellular membranes (for example, nerve and muscle at the synapse, endothelium and podocyte in the glomerulus), whereas BLs poor in s-laminin separate cells from interstitial connective tissue[12].

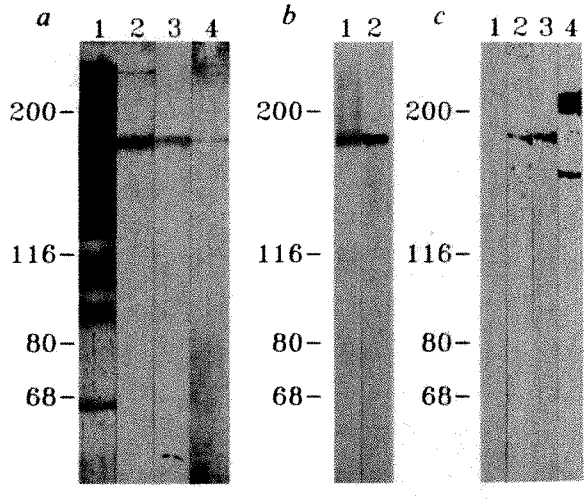


FIG. 2 Identification of s-laminin by immunoblotting. *a*, A crude extract of renal cortex was analysed by gel electrophoresis and either silver-stained (lane 1) or transferred to nitrocellulose and probed with mAbs C4 (lane 2), D5 (lane 3), or D7 (lane 4). Positions of relative molecular mass standards are indicated in thousands; the M_r of s-laminin is ~190K. *b*, Peak fraction from chromatofocusing column, silver-stained (lane 1) or immunoblotted with mAb C4 (lane 2); compare with lanes 1 and 2 in *a*. *c*, Renal extract, prepared as in *a*, and immunoblotted with normal serum (lane 1), a polyclonal antiserum to a fusion protein encoded by s-laminin cDNA RK65-6 (lane 2), mAb D5 (lane 3), or a polyclonal antiserum to laminin (lane 4).

METHODS. Sample preparation and electrophoresis were performed as described[45], using 5% polyacrylamide gels. Electropheretic transfers to nitrocellulose (S&S BA85) were as described[46]; transfers were immunostained with 1:1,000 dilutions of anti-s-laminin antibodies or rabbit anti-laminin serum (Polysciences) and alkaline phosphatase-second antibody (1:1,500; Boehringer), according to the manufacturer's instructions. Transferred relative molecular mass markers were stained with 0.2% Ponceau S (Eastman Kodak) in 3% trichloroacetic acid (Aldrich) and 3% sulphosalicylic acid (Sigma). To purify s-laminin, homogenates of renal cortex were successively treated with 2M $MgCl_2$, 3% NP-40 and 6 M guanidine-HCl to remove soluble, membrane-bound and chaotrope-soluble components. S-laminin was then extracted from the residue with 6 M guanidine-HCl plus 10 mM dithiothreitol, resulting in a purification of ~100-fold. After dialysis, chromatofocusing chromatography was performed on PBE 94 (Pharmacia), following the manufacturer's instructions. Briefly, BL extract was dissolved in 7 M urea (ICN-Schwarz Mann) plus 5% β-mercaptoethanol, 2% NP-40, 25 mM imidazole pH 7.4, applied to a 1 cm by 10 cm column equilibrated with the same solution, and eluted with 7 M urea, 5% β-mercaptoethanol, 2% NP-40, 12½% polybuffer 74 (Pharmacia) pH 4.0. Fractions were assayed for s-laminin by immunoblotting and for protein content by dye binding (Bio Rad). S-laminin was eluted at a pH of ~6.5. To prepare antisera, RK65-6 was recloned into the pET vector[40], *Escherichia coli* were transformed and the protein was purified from a cell lysate by gel electrophoresis; gel slices containing 0.5 mg of fusion protein were homogenized in RIBI adjuvant and injected into rabbits.

Identification of s-laminin

Because glomerular BL has been characterized in detail[13], we used kidney to identify the antigen(s) recognized by mAbs C1, C4, D5 and D7. BL-rich fractions were analysed by immunoblotting. When samples were not reduced, the antibodies recognized only material with a relative molecular mass (M_r) of more than 10^6 (1,000K). Under reducing conditions, however, C4, D5 and D7 all stained a band of apparent M_r ~190K (Fig. 2*a*). C1 did not stain immunoblots, but C1-agarose precipitated C4-reactive material of about 190K. Immunoblots of two-dimensional gels (data not shown) and chromatofocusing chromatography (Fig. 2*b*) demonstrated that these mAbs recognized the same protein; it has an isoelectric point of ~6.5. Thus, a ~190K protein is identified as a subunit of s-laminin. In addition, C4 and D7 weakly stained a band of M_r ~400K; we have not studied the relationship of the ~400K material to the ~190K s-laminin.

The insolubility of s-laminin confirmed its association with the extracellular matrix. Little immunoreactive material was released upon incubation of s-laminin-rich fractions with high salt solutions, non-ionic detergents, reducing agents or strongly chaotropic agents. Extraction with a chaotrope (guanidine-HCl) plus a reducing agent (dithiothreitol) did, however, release most of the s-laminin detectable by immunoblotting. Collagenase treatment did not affect the M_r of extracted s-laminin. Solubil-

FIG. 3 *a*, Alignment of cDNA clones which encode s-laminin. The location of clones isolated from rat kidney (RK) and muscle (RM) λgt11 libraries are shown on a schematic sequence. Loops represent introns found in RK65-3. Insert indicates immunoreactivities of fusion proteins generated from cDNAs with mAbs described in the text. *b*, Nucleotide and deduced amino-acid sequence of s-laminin. Predicted domains (see text), and their respective residues, are VI (1–248), V (249–520), IV (521–749), III (750–1,158), II (1,159–1,377), α (1,378–1,410) and I (1,411–1,766). Residues identical to those in mouse laminin B1[18] are underlined. The seven potential *N*-glycosylation sites (laminin B1 contains 14; see ref. 18) are in bold face. A predicted signal peptide of 35 amino acids (residues −35 to −1) is similar to that of laminin B2. If the signal peptidase cleavage site is located after the sequence Thr-Leu-Ala, in agreement with the '−3, −1' rule[47], s-laminin begins with glutamine, as do human and mouse laminin B1, human laminin B2 and mouse laminin A (refs 18, 19, 21, 23). A polyadenylation site (underlined) precedes the poly(A) tail by 12 bp.

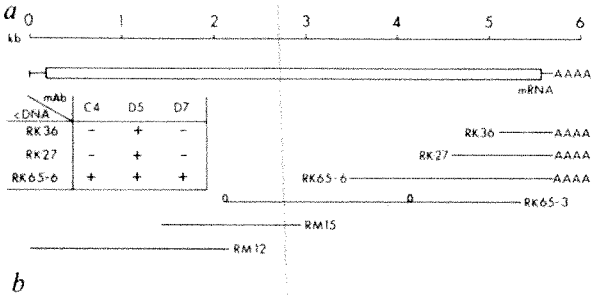
METHODS. RK27 and RK36 were isolated from a kidney expression library (Clontech) using anti-s-laminin antibodies at 1:1,500 dilutions and 1:2,000 dilutions of an alkaline phosphatase-goat anti-mouse IgG, using published procedures[48]. RK65-3 and RK65-6 were isolated from the same library by hybridization at 42 °C with a ^{32}P-labelled fragment of RK27, as previously described[36]; washes were at 65 °C in 0.1 × SSC containing 0.1% SDS. RM12 and RM15 were isolated from a λgt11 library constructed from rat muscle poly(A)$^+$ RNA as previously described[49], using a ^{32}P-labelled fragment of RK65-3 (with washes as above) and a ^{32}P-labelled octadecanucleotide near the 5′ end of RK65-3 (with washes at 58 °C in 6 × SSC containing 0.1% SDS). The nucleotide sequence was determined after subcloning into Bluescribe (Stratagene), with a modification of the dideoxy chain termination method[50], using Sequenase (USB), oligonucleotide sequencing primers (synthesized at the Protein Chemistry Laboratory of Washington University) and α-[^{35}S]-dATP (NEN) as the label. All clones shown were sequenced on both strands.

ized s-laminin bound to concanavalin A-agarose and wheat-germ agglutinin-agarose and could then be eluted by appropriate hapten sugars. These data indicate that s-laminin is a non-collagenous glycoprotein tightly attached to BL by a combination of hydrophobic and disulphide bonds.

Neuronal adhesion to s-laminin

As a first step in testing the hypothesis that regenerating axons interact with s-laminin at synaptic sites, we tried to determine whether neurons adhere to this protein. Neurons were incubated in s-laminin-coated tissue culture dishes for 2 h at 37 °C; unbound neurons were then rinsed away and adherent neurons counted. Chick ciliary neurons were chosen for these assays because they innervate striated muscle *in vivo*[14] and recognize synaptic sites on adult muscle fibres *in vitro*[15].

Neurons adhered comparably well to laminin and to a preparation of s-laminin that had been purified ~2,000-fold from renal cortex by selective extraction and chromatofocusing (Table 1, series A). Several other proteins were not adhesive in this assay. The s-laminin preparation contained no detectable laminin or



fibronectin (as judged by immunoblotting and silver-staining of SDS gels), but was not homogeneous. These results indicate that neurons adhere to s-laminin, but do not exclude the possibility that contaminants in the s-laminin preparation are involved. Neurons also adhered to s-laminin prepared by hydroxylapatite or mAb D5-agarose chromatography (Table 1, series A) however. Thus, neurons do adhere to s-laminin or to molecules that co-purify with s-laminin by three different methods.

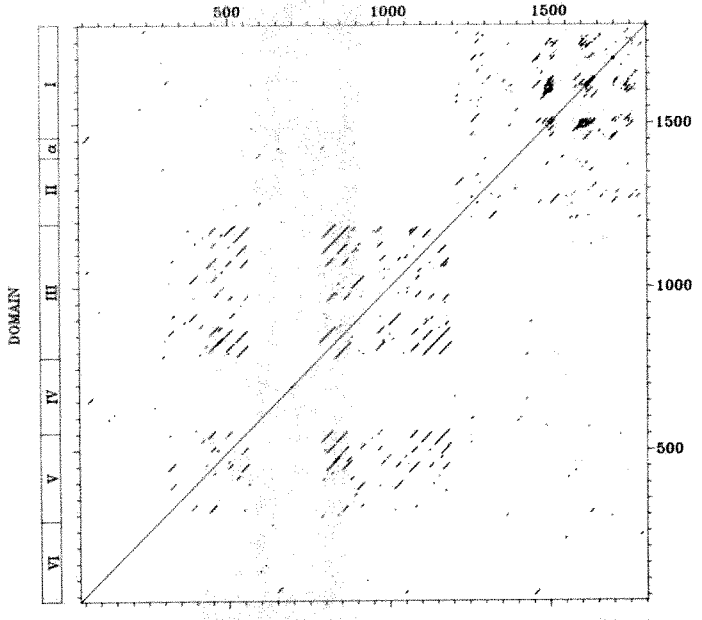
Cloning of s-laminin cDNA

We used molecular cloning to obtain structural information about s-laminin and a source of recombinant protein for further assays of function. A rat kidney cDNA library in the expression vector λgt11 was screened with seven mAbs to s-laminin (the four described above and three others). From 7×10^6 plaques, we identified two clones that reacted with mAb D5 (RK27 and RK36; Fig. 3*a*). Using a fragment of RK27 as a probe, clones RK65-3 and RK65-6 were isolated by screening an additional 1.5×10^6 plaques. A 5′ fragment of RK65-3 was then used to screen 1.5×10^6 plaques from a rat muscle cDNA library; 19 clones, including RM12 and RM15, were isolated.

Sequence analysis indicates that these cDNAs span a distance of 5.6 kilobases (kb), comprising 68 base pairs (bp) of 5′ untranslated region, a single open reading frame of 5,403 bp, a 3′ untranslated region of 110 bp and a poly(A) tail (Fig. 3*b*). Monoclonal antibodies C4, D5 and D7 all bound specifically to a fusion protein encoded by RK65-6 (see Fig. 3*a*), indicating that the cDNA-encoded protein shares three distinct epitopes with s-laminin. Furthermore, antisera to RK27 and RK65-6 fusion proteins stained synaptic sites in sections (data not shown) and recognized s-laminin on immunoblots (Fig. 2*c*). Sequences of cDNAs derived from muscle and kidney are identical in regions of overlap. On northern blots, RK65-6 hybridized to a single mRNA of ~5.7 kb in RNA from kidney and muscle (data not shown). Together, these results demonstrate that we have cloned virtually the entire s-laminin mRNA.

S-laminin mRNA encodes a protein with a predicted M_r of 196,472 that is rich in cysteine, has seven potential *N*-glycosylation sites and includes an *N*-terminal signal sequence of $M_r \approx$ 3,471 characteristic of membrane-spanning and secreted proteins (Fig. 3*b*). These features are consistent with the properties of s-laminin described above. The most striking feature of the s-laminin sequence, however, is its homology to the laminin subunits. Laminin is composed of three subunits, A, B1 and B2, arranged in a cruciform structure[16]. On the basis of their predicted secondary structures, the laminin subunits have been divided into several domains. Domains I and II contain heptad repeats thought to form coiled-coil α-helices; III and V contain ~50-residue-long cysteine-rich repeats postulated to form loops; and IV and VI are predicted to be predominantly globular[17–24]. In the B1 but not the A or B2 subunits, domains I and II are separated by a short cysteine-rich domain, called α[18]. S-laminin contains all seven domains, I–VI plus α; matrix analysis highlights the repeats and the borders between domains (Fig. 4*a*). S-laminin has more similarity with the B1 subunit of laminin than with A and B2; all of the subunits are related to each other (Fig. 4*b*).

Several regions of the laminin B1 molecule have been implicated in functional interactions. The sequence YIGSR (in the one-letter code) in domain III adheres to cells of several types[25]; s-laminin contains the sequence YTGLR in a corresponding position. A 20-mer from domain IV



		VI	V	IV	III	II	α	I	TOTAL
S–LAMININ VS.	MOUSE B1	72	65	42	60	26	52	38	52 (68)
	HUMAN B1	71	65	40	60	26	49	39	51 (68)
	DROSOPHILA B1	58	56	28	49	27	61	23	41 (56)
	MOUSE B2	22	40	13	41	13	–	17	25 (46)
	MOUSE A	20	36	14	38	16	–	15	24 (41)
MOUSE B1 VS.	HUMAN B1	99	92	93	94	89	91	92	93 (96)
	DROSOPHILA B1	59	54	28	56	20	42	25	41 (58)
	MOUSE B2	21	39	10	40	14	–	16	25 (45)
	MOUSE A	21	35	12	38	17	–	17	25 (40)

FIG. 4 *a*, Dot matrix plot comparing predicted amino-acid sequence of s-laminin to itself, to demonstrate internal repeats. Dots indicate positions at which more than 16 amino acids in a stretch of 30 are identical. Numbering of residues is from Fig. 3. Borders between domains are indicated along the axes. Multiple repeats in domains I–III and V are evident. *b*, Homologies (percentage identity) between amino-acid sequences of s-laminin and the subunits of laminin. Values of ≥35% are boxed. Sequences and their divisions into domains are from refs 17–24. Homologies were computed using the UWGCG GAP program with gap penalty = 0.3. Gap weight = 1 for *Drosophila* α and 5 for all other comparisons. For laminin A, domain G was omitted and scores for domains III a and b were averaged, as were scores for IV a and b. Values in parentheses under "total" are percentage similarity, in which evolutionarily conservative substitutions are given some weight. Note that s-laminin and laminin B1 are equally homologous to the *Drosophila* sequence that has been termed B1.

TABLE 1 Adhesion of ciliary neurons to s-laminin

Substrate		Neurons per field
Series A		
Laminin		21 ± 1
s-laminin	Chromatofocusing peak	15 ± 1
	Hydroxylapatite peak	24 ± 2
	D5-agarose eluate	20 ± 1
Bovine serum albumin		3 ± 1
Series B		
Laminin	+mAb C14	25 ± 2
	+mAbs C4 + D5	22 ± 2
		23 ± 3
Recombinant s-laminin (pET-RK65-6)		29 ± 3
	+mAb C14	25 ± 6
	+mAbs C4 + D5	3 ± 0

Laminin (Collaborative Research; 0.1 mg ml^{-1}), purified s-laminin 0.01 mg ml^{-1}), recombinant s-laminin (0.01 mg ml^{-1}), or bovine serum lbumin (BSA) (Sigma; 30 mg ml^{-1}) were applied to nitrocellulose-coated ssue culture dishes as described[43]; free sites on the nitrocellulose were ıen blocked with 30 mg ml^{-1} BSA for 2 h. Neurons dissociated from embry-nic-day-10 chick ciliary ganglia[15] were suspended in medium (DMEM with . mg ml^{-1} BSA and 10 mM HEPES, pH 7.3) and added to the dishes. After 2 h of incubation at 37 °C, plates were rinsed twice with medium, adherent ells were fixed with 2% formaldehyde and 2% glutaraldehyde and neurons vere counted. Wells containing antibodies (20 μg ml^{-1}) were incubated for .5 h before the addition of neurons. Each microscope field covered an area of 3 mm^2. Mean ± s.e.m. from 3–12 experiments are shown. Chromatofocus-ng is described in the Fig. 2 legend and illustrated in Fig. 2*b*. For chromatogra-phy on hydroxylapatite, immunoreactive material was eluted from a Bio-Gel HPHT (Bio-Rad) column with a gradient of sodium phosphate (10–350 mM, n 4M urea, 0.1% β-mercaptoethanol and 10 mM $CaCl_2$, pH 6.9). For immuno-affinity purification, mAb D5 was coupled to Affigel HZ (Bio-Rad); s-laminin was eluted with 0.1 M glycine-HCl, pH 2.8. Non-s-laminin polypeptides promi-nent in the hydroxylapatite and D5-agarose eluates were different from those detectable by silver staining in the chromatofocusing peak. Preparation of s-laminin fusion proteins in the pET vector[40] will be described elsewhere (D.D.H., B. Porter, J.P.M. and J.R.S., in preparation). Neurons did not adhere at levels above background to a non-s-laminin fusion protein or to a pET vector control. C14 is a mouse monoclonal antibody that recognizes a non-s-laminin BL antigen[6].

(RYVVLPRPVCFEKGMNYTVR) inhibits adhesion of some cells and binding of heparin to laminin[26]; s-laminin is identical in 11 of 20 positions (RVLVFPRPVCLEPGLSYKLK). A hexapeptide from domain V (LGTIPG) inhibits binding of a cellular receptor to laminin (R. Mecham, personal communication); the sequence of s-laminin is RGTVPG in the corresponding position. Finally, a neurite outgrowth-promoting site on laminin has been localized to a region near the C terminus of B1 and/or B2[27,28]; this domain is one of the least conserved between s-laminin and the laminin subunits (Fig. 4*b*). We do not yet know whether these differences conserve or abolish adhesive function.

Database searches (Genbank and NBRF) did not reveal any striking similarities of s-laminin with proteins other than laminin, although short regions of homology between several proteins and the repeats in domains I–III and V were detected, as noted previously for laminin[19–22]. More detailed comparisons failed to reveal extensive homologies between s-laminin and other neuroactive proteins that are associated with BLs under some circumstances—fibronectin[29], entactin[30], collagen IV[31], NCAM[32], L1[33] and tenascin[34]. S-laminin is also distinct from other proteins concentrated at the neuromuscular junction—acetylcholine receptor subunits[35], the 43K RAPsyn protein[36], acetylcholinesterase[37] (or its collagenous subunit[38]), or agrin[39] (the agrin N-terminal sequence was communicated by M. Smith and U. J. McMahan). The possibility that s-laminin is the rat equivalent of laminin B1 can be dismissed because of the limited amino-acid sequence identity (Fig. 4*b*) and because a laminin B1 cDNA (obtained from B. Hogan; see ref. 18) and a s-laminin cDNA encoding equivalent domains (I and II) recognize distinct restriction fragments on Southern blots of genomic DNA (data not shown). Thus, s-laminin is apparently a hitherto undescribed component of BL.

Neuronal adhesion to recombinant s-laminin

The cDNA clone RK65-6 encodes the C-terminal 40% of s-laminin. This sequence was subcloned into the pET expression vector[40] to produce a protein consisting of an 80K s-laminin fragment fused to a 10 amino-acid phage peptide. When tested in the cell-binding assay described above, this fusion protein mediated adhesion of ciliary neurons to substrata; adhesion was blocked by mAbs C4 and D5 (Table 1, series B). Thus, s-laminin contains at least one site that neurons recognize.

Conclusions

Starting with an antiserum that stained synaptic sites in muscle[5], we have identified a laminin-like component of synaptic BL with which motoneurons can interact. We undertook this work hoping to find factors that regenerating motor axons recognize at original synaptic sites, and our results support the candidacy of s-laminin for this role. The availability of s-laminin cDNAs will now allow critical tests of this hypothesis: effects of recombinant s-laminin on neuronal behaviour can be assessed and antisera to recombinant s-laminin can be tested for their ability to perturb axonal recognition of synaptic BL *in vitro*[15] and *in vivo*. In addition, recombinant s-laminin, and antisera to it, will be useful reagents for probing the function of s-laminin in non-muscle BLs.

The homology of s-laminin to laminin is interesting in two respects. First, it provides evidence for the existence of a laminin gene family that extends beyond the genes for the three laminin subunits. Some other extracellular matrix molecules (for example, the collagens), and some of their cellular receptors (for example, the integrins) are encoded by families of related genes[41,42], and the specificity of cell–matrix interactions may result in part from the differential expression of individual members of such families. In this context, it will be important to determine whether s-laminin-like homologues of laminin A and/or B2 subunits exist, whether other laminin-like genes exist, whether s-laminin can substitute for laminin subunits to form chimaeric complexes and whether any effects previously attributed to laminin are actually mediated by s-laminin. Second, it has recently become clear that laminin is a major axon outgrowth-promoting component of extracellular matrices in the peripheral nervous system and that neurons have several laminin receptors[9]. The results presented here raise the possibility that laminin itself is but one of a group of related molecules that could act together to regulate axonal behaviour in complex ways. In muscle in particular, laminin-rich pathways might provide a favourable terrain for regenerating axons to follow, whereas s-laminin at original synaptic sites might direct axons to stop growing and/or to differentiate into nerve terminals. □

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LETTERS TO NATURE

Submillisecond optical pulsar in supernova 1987A

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WE have detected an optical pulsar with frequency f = 1,968.629 Hz at the location of supernova 1987A in the Large Magellanic Cloud. The brightness of the pulsed light increased from magnitude 19 to 18 during a 7-h observation period starting on 18.1 January 1989 UT, in observations taken using the 4-m telescope at Cerro Tololo Inter-American Observatory. The frequency of the pulsar during this same period varied in a nearly sinusoidal manner, with an amplitude of 1.5×10^{-3} Hz and a period of 8 h. If this is interpreted as the result of a binary orbit, it suggests that there may be a Jupiter-sized object orbiting the pulsar at a distance of 10^6 km. No significant pulsations were seen in data taken with the same detection system at the 100″ telescope at Las Campanas Observatory on 31 January UT, indicating that the pulsar had dropped below our detection limit of magnitude 20. Significant second ($2f$) and third ($3f$) harmonics were also observed.

The detection of the optical pulsar[1] in the supernova 1987A represents the culmination of a search program begun in March 1987, consisting of 37 separate observations from three different observatories[2]. A detector was used that was designed to allow for a pulsar search in the background of the bright light of the supernova. In the detector system, the current from a silicon photodiode was integrated over an interval of 2×10^{-4} s, digitized and written to tape. Manufacturer specifications for the photodiode, Hamamatsu model S1188-06, report the quantum efficiency of the silicon photodiode peaking at 70% at 0.6 μm, and greater than 50% between 0.4 μm and 0.9 μm; it averages about 60% over this range. No filter was used for the observations. The aperture size was 13 arcsec.

The analysis of the data follows that of Middleditch and Cordova[3]. We use a modified Fourier analysis[4], which differs from the standard Fourier transform by allowing the period of the expected signal to change with time, because the frequency of a pulsar may change significantly during an observation. We then searched the resulting power spectrum for nonstatistical peaks in the frequency range from 0.003 Hz to the Nyquist limit of 2,500 Hz (half the sampling frequency).

The data for 18 January were split into 15 independent contiguous sets. Except for three incomplete data sets, each contains 8 million (2^{23}) 200-μs samples and covers just under $\frac{1}{2}$ hour (1,677 s). Each set was analysed independently to find the frequency and frequency derivative that maximize the peak in the power spectrum. The results are listed in Table 1, which also includes the power found in the aliased second ($2f$) and third ($3f$) harmonics. The times given are the midpoints of each data set. The peak frequencies are shown as a function of time in Fig. 1*a*; shown in Fig. 1*b* are the same data after correction

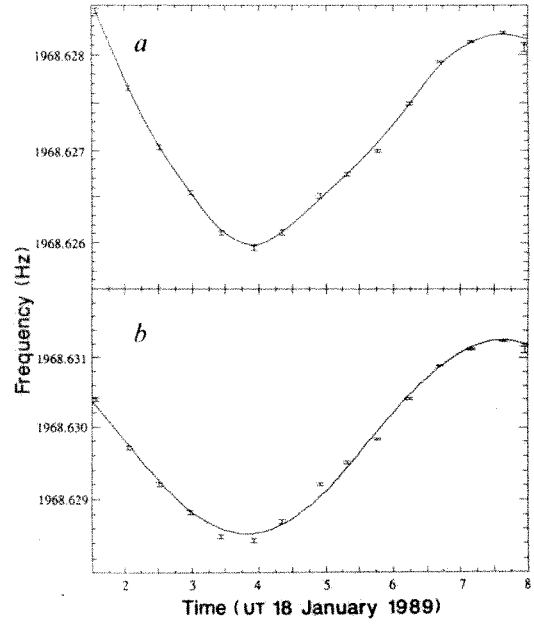


FIG. 1 *a*, Observed frequency versus time, for the 7-h run on 18 January 1989. The solid line is a guide to the eye. *b*, Same data, corrected for the Doppler shift resulting from the rotation of the Earth and its motion around the Sun (barycentric coordinates). The solid line is a sine function with best-fit values for amplitude, frequency, phase and offset.

TABLE 1 Observational data for the SN1987A pulsar

UT (h)	Frequency*	δf†	df/dt‡	Power§	σ‖	2f power¶	σ	3f power	σ
1.57	1,968.630387	25	−5.4	1,148	13	3,948	7		
2.04	1,968.629710	24	−4.2	1,334	14	3,417	6		
2.51	1,968.629197	27	−3.1	941	12	3,977	7		
2.97	1,968.628823	25	−2.2	1,176	13				
3.43	1,968.628496	25	0.36	1,058	13	1,676	4		
3.92	1,968.628438	28	0.75	903	11				
4.34#	1,968.628700	32	1.4	830	13	3,877	7		
4.90#	1,968.629206	25	0.5	1,548	19	4,094	7		
5.32	1,968.629513	17	2.0	2,188	18	8,689	9		
5.77	1,968.629833	16	3.1	3,049	21	6,042	8	19,235	6.1
6.25	1,968.630407	14	4.6	4,250	25	7,564	9		
6.70	1,968.630888	11	1.6	7,191	31	24,158	15	14,457	5.5
7.16	1,968.631135	10	1.8	8,399	34	31,914	18	10,835	4.9
7.64	1,968.631253	9	−1.3	9,745	37	43,528	21	31,069	8.2
7.95#	1,968.631121	48		3,675	20	7948	9	19,677	6.1

* In Hz, barycentric coordinates. † Statistical uncertainty in the frequency determination, in units of 1×10^{-6} Hz. ‡ In 10^{-7} Hz s^{-1}. § Spectral power. The signal power is proportional to the square root of this. ‖ Number of standard deviations, indicating the statistical significance of the spectral power in the previous column. ¶ Spectral power for the second and third harmonics was calculated by compensating for the losses from aliasing and from the response of the front-end amplifier for the photodetector. # Values of power are low because data did not fill entire interval.

for the Doppler shift resulting from the rotation of the Earth and its motion around the Sun. The error bars shown are $\pm1\sigma$, determined by the run duration and the power in the peak. After the Doppler correction, the modulation exhibits a sinusoidal behaviour. A least-squares fit to a sine curve gave a solution with an amplitude of 1.36×10^{-3} Hz, a period of 7.6 h and a central frequency of $f_0 = 1{,}968.6299$ Hz; this fit is shown as the solid line in Fig. 1*b*. Also allowing a constant frequency derivative $(df/dt)_0$ in the fit gives an amplitude of 1.77×10^{-3} Hz, a period of 8.8 h, and $(df/dt)_0 = -5.3\times10^{-8}$ Hz s^{-1}. We conclude that the period of the modulation is 8 ± 1 h, the amplitude is $(1.5\pm0.3)\times10^{-3}$ Hz, $-(df/dt_0)<10^{-7}$ Hz s^{-1} and $(dP/dt)_0<3\times10^{-14}$ s s^{-1}. The residuals after the two fits were $(6\text{–}8)\times10^{-5}$ Hz (r.m.s.); these are statistically significant when compared with uncertainties in frequency (typically $(1\text{–}3)\times10^{-5}$ Hz).

Twelve minutes after the end of the 7-h observation of SN1987A, the telescope was pointed at the globular cluster NGC3201 (RA $10^h\,17^m$, δ −46°) for a half-hour of observation. No significant periodic signal was found in the globular-cluster data. We note that the pulsar signal was strongest just before these globular-cluster observations.

Each of the 8-million-point transforms showed a significant signal at the aliased frequency for the second harmonic, and five transforms (taken near the end of the observation, when the pulsar was brightest) showed a significant signal at the aliased frequency of the third harmonic. The frequency and intensity of the harmonics were modulated in the same manner as the fundamental, if one takes into account the frequency inversion expected for the aliased signals. The phase coherence of the pulsations was such that they could be tracked to an integral number of cycles across the individual data sets.

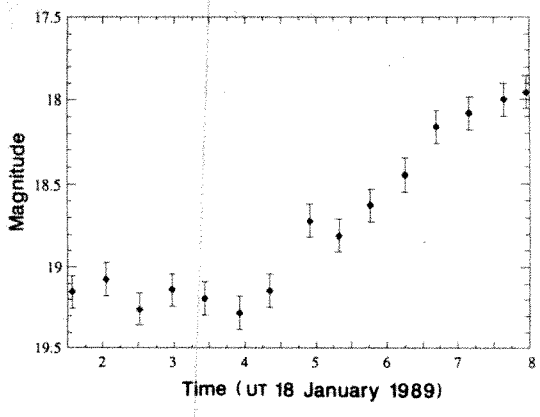


FIG. 2 Magnitude versus time for the 1,968-Hz fundamental frequency, for the same run as in Fig. 1. In addition to the uncertainties shown, there may be a systematic uncertainty of ±0.5 magnitude for each point.

We calculate the magnitude of the pulsar by comparison with a calibration run on the Crab pulsar (PSR0531 + 21) performed one year earlier, on 18 January 1988. We find a broad-band magnitude varying slowly over 7 h from mag 19 to 18 (Fig. 2), with a very significant rise in brightness from the beginning to the end of the run. The maximum magnitude observed was seven magnitudes dimmer than that of the supernova (which was magnitude 11 on 18 January); this is roughly the same as the magnitude difference between the Crab pulsar (magnitude 16.6) and the Crab nebula (magnitude 8.6). Despite a threefold increase in brightness, the ratio of the power emitted in the three harmonics is constant to within 40%. The ratio of amplitudes of the first three harmonics, for the run at 7.64 UT (the run with the best statistical significance) was 1 : 1.8 : 1.6. For comparison, in a short run on the Crab pulsar we observed the amplitudes of the first three harmonics to be in the ratio 1 : 1.8 : 1.1. Thus the signal shape is basically similar to the double-peaked light curve of the Crab.

It appears unlikely that we are observing two pulses per rotation of a pulsar whose rotation frequency is 984 Hz. No subharmonic was present at a level greater than 3% of the amplitude of the fundamental. If there were two pulses per rotation, they would have to be separated by exactly half of the rotation period and their shapes would have to be the same.

In the 36 runs made previous to the observation of the pulsar[2], the typical 90% confidence upper limit on the fraction of optical light that is pulsed is 2×10^{-4} of the total light from the supernova. The 90% figure is based on the lack of any peaks in the power spectrum more than 27.3 times the average local spectral power level S_{loc} at nearby frequencies; typically, the highest peaks actually observed were 20 times S_{loc}. (The probability that a random fluctuation will yield a peak with spectral power S_1 is $\exp(-S_1/S_{loc})$.) The range of these limits is set by the observation conditions and the noise at particular frequencies. Before January 1989, our faintest limit was $m = 20.6$, made on 27 August 1988, also at CTIO. On the evening of the detection, the observed spectral power for the fundamental in the 8-million-point transforms was $S_1 = 66\times S_{loc}$ at its weakest, and as much as $S_1 = 699\times S_{loc}$ at its strongest. The statistical significance of the peaks corresponding to these spectral power levels is shown in Table 1, and varies from 11 to 37 standard deviations. Thus each of the 15 runs taken on 18 January showed a signal of much greater significance than had been seen on any of the previous 36 runs over the preceding two years.

Given that the magnitude changed from 19 to 18 over the 7-h period on 18 January, it is not too surprising (although disappointing) that the brightness dropped below our detection threshold of 20th magnitude on 31 January. It is possible that the observed brightness variation was due to obscuring material in the expanding supernova shell.

In view of the lack of confirmation so far of the pulsations reported here, it seems useful to summarize the evidence for their proposed origin. They are extremely strong, and are clearly real; the question is whether they originate in the supernova or are merely instrumental. They are unlike anything seen in 36 previous runs using the same instrumentation over the preceding two years; a number of artefacts in the data have been seen over this period, but these pulsations are unprecedented in their strength and their behaviour. Taking into account the 8-h sinusoidal variation, they are phase-coherent over the entire 7 h of observation, so that an integral number of cycles for the fundamental frequency can be counted unambiguously between sets of data. The pulsations are as narrow in $(f, \mathrm{d}f/\mathrm{d}t)$ space as would be a δ-function for such a discrete Fourier transform, which implies an extremely good time-keeping mechanism. In addition to the fundamental frequency, evidence is seen for two higher harmonics which track the fundamental in frequency and intensity. The pulsations are not seen in data taken on another object immediately following the supernova observation. The observed frequency modulation is a good fit to a sine wave only when transformed to barycentric coordinates. The ratio of harmonics is similar to that seen in the Crab pulsar.

With a 0.51-ms period, the pulsar is spinning three times faster than any other known pulsar. For a nominal neutron-star radius of 10 km, the velocity of the surface is 40% of the speed of light. The radius of the light cylinder is only 24 km, as opposed to 1,600 km for that of the Crab pulsar. Assuming a nominal mass of 1 $M_\odot$, a uniform density and a spherical shape, the rotational energy is 6×10^{52} erg, which is comparable to the entire energy output from the explosion of SN1987A (the shape would, however, be highly non-spherical, because the centrifugal force is comparable to the gravitational force). This rotational energy, together with our limit on the period derivative ($\mathrm{d}P/\mathrm{d}t < 3\times10^{-14}$ s s^{-1}) means that $\mathrm{d}E/\mathrm{d}t < 7\times10^{42}$ erg s^{-1}.

The small value of $\mathrm{d}P/\mathrm{d}t$ suggests that this pulsar is still very close to its initial frequency. Friedman and coworkers[5,6] have shown that the very existence of a submillisecond pulsar rules out most of the equations of state that have been proposed for neutron stars. Only a very soft equation of state allows the high central compression of matter near the core of the star that is required to provide the gravitational binding necessary to overcome the centrifugal force.

Even a small deviation from axial symmetry in the mass distribution would yield considerable gravitational radiation. If we assume an asymmetric element of mass δm on the surface, yielding a quadrupole moment Q that changes with the rotation of the pulsar, then the energy released as gravitational radiation is approximately

$$-\frac{\mathrm{d}E}{\mathrm{d}t} \approx \frac{G\omega^6 \delta m^2 R^4}{45c^5} = 10^{54}(\delta m/M_\odot)^2 \text{ erg s}^{-1}$$

where ω and R are the angular frequency and radius of the pulsar, respectively. Our limit of $-\mathrm{d}E/\mathrm{d}t < 7\times10^{42}$ erg s^{-1} gives a limit of $\delta m < 3\times10^{-6}\,M_\odot$. Compared with the Crab pulsar, the gravitational radiation expected on the Earth from comparable mass irregularities is greater by a factor of $(\omega/\omega_{\mathrm{Crab}})^6/(D/D_{\mathrm{Crab}})^2$, that is, 10^8 times larger. Thus the pulsar may be a candidate for the detection of gravitational radiation, although for maximum sensitivity the detection must take into account the modulated frequency behaviour, perhaps by using an ephemeris derived from the optical signal.

If we assume that the energy loss rate from the pulsar is less than that radiated by the supernova ($\sim10^{38}$ erg s^{-1}), then we conclude that the pulsar must have a relatively weak magnetic field B. This can be seen easily by comparison with the Crab pulsar, which coincidentally also loses power at the rate of 10^{38} erg s^{-1}. If we use the scaling law for dipole emission with radiated power proportional to $B^2\omega^4$, the magnetic field would be lower than that of the Crab pulsar by the square of the frequency ratio, $(1{,}968/30.2)^2 = 4\times10^3$. Taking $B_{\mathrm{Crab}} = 4\times10^{12}$ gauss, we find the field for the SN1987A pulsar to be $B = 10^9$ gauss.

The observed 8-h frequency modulation of the pulsar could be due to an intrinsic change in the rotation frequency of the pulsar, or to a Doppler shift. If the change is intrinsic, it could be due to a periodic change by 10^{-6} in the moment of inertia of the pulsar, or to oscillations between two components of the pulsar (such as a hard crust and a superfluid core). In the two-component model, the rotation angle between the two components oscillates with a period of 8 h and an amplitude of $(1.4\times10^{-3}$ Hz$)\times(8$ h$) = \pm40$ radians $= \pm6.4$ turns, that is, about 13 turns peak-to-peak. Because of the great amount of energy stored in the pulsar spin, the forces required to drive such oscillations are enormous. A successful model must account for the relatively long period of 8 h while maintaining sufficient linearity under 13 turns to reproduce the observed sine-like variation.

The modulation could also be explained as a Doppler shift resulting from motion of the pulsar in a binary system. The observed frequency modulation is similar to that seen in PSR1957+20, a 1.5-ms pulsar with a binary companion in a 9.16-h orbit[7]. The frequency modulation of 1.4×10^{-3} Hz for the SN1987A pulsar implies a velocity (in km s^{-1}) of $0.2/\sin i$, where i is the inclination angle. The nearly sinusoidal behaviour implies a nearly circular orbit; the orbital frequency is $\Omega_0 = 2.2\times10^{-4}$; the orbital radius of the pulsar is $r_0 = v/\Omega_0 = (10^3$ km$)/\sin i$. From Kepler's law, the distance between the pulsar and its companion is $(GM/\Omega_0^2)^{1/3} = 1.3\times10^6$ km. The mass of the companion is then $10^3/(1.3\times10^6 \sin i) = 8\times10^{-4}\,M/(\sin i)$, which is comparable to the mass of Jupiter for $M = M_\odot$. The mass function is thus about $2.5\times10^{-10}M_\odot$.

This orbit would have been well within the radius of the blue giant progenitor star; it could not have survived the supernova explosion in such an orbit, so it would have to have been placed into orbit with the pulsar after the explosion. Perhaps it was left over from the core collapse, or ejected from the spinning pulsar, and then circularized by nearby orbiting material. We note that the pulsar has almost the maximum possible rotational velocity, and that the angular momentum of the companion is similar to that of the pulsar. The deviations of the oscillations from sinusoidal are statistically significant, and may indicate departures from a circular orbit, perhaps as a result of additional debris orbiting the pulsar, or they may indicate intrinsic changes in the pulsar rotation rate. The evolution of the sinusoidal modulation will be of great interest as a possible probe of the region 10^6 km from the pulsar.

Note added in proof. The pulsar was not seen in later observations on 13 February (Siding Springs 2.3-m telescope), and 16, 20 and 21 February (Las Campanas 100″ telescope), all with limits fainter than 20th magnitude. □

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A numerical experiment on the chaotic behaviour of the Solar System

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LAPLACE and Lagrange made an essential contribution to the study of the stability of the Solar System by proving analytically that, to first order in the masses, inclinations and eccentricities of their orbits, the planets move quasiperiodically. Since then, many analytic quasiperiodic solutions have been sought to higher order[1–10]. I have recently constructed an extensive analytic system of averaged differential equations containing the secular evolution of the orbits of the eight main planets, accurate to second order in the planetary masses and to fifth order in eccentricity and inclination, and including corrections from general relativity and the Moon[8–10]. Here I describe the results of a numerical integration of this system, extending backwards over 200 million years. The solution is chaotic, with a maximum Lyapunov exponent that reaches the surprisingly large value of ~1/5 Myr^{-1}. The motion of the Solar System is thus shown to be chaotic, not quasiperiodic. In particular, predictability of the orbits of the inner planets, including the Earth, is lost within a few tens of millions of years. This does not mean that after such a short timespan we will see catastrophic events such as a crossing of the orbits of Venus and Earth; but the traditional tools of quantitative celestial mechanics (numerical integrations or analytical theories), which aim at unique solutions from given initial conditions, will fail to predict such events. The problem of the stability of the Solar System will have to be set up again, and the qualitative methods initiated by Poincaré definitely need to replace quantitative methods in this analysis.

After the fundamental work of Laplace and Lagrange, it was assumed that the motions of the planets were quasiperiodic, and efforts in celestial mechanics in the nineteenth century were devoted to the construction of quasiperiodic solutions for all the bodies of the Solar System using perturbation techniques. This approach was questioned when Poincaré proved that, if it is possible to expand formally the solutions of the planet motions in infinite series, then in general these series are not convergent[11,12].

The KAM theory[13] nevertheless proved that if the perturbation is not too large (that is, if the masses, eccentricities and inclinations of the planets are sufficiently small), then a large set of initial conditions leads to quasiperiodic solutions[13]. It was not proved that the Solar System fulfilled these requirements. In fact, the Solar System is much too complicated, with far too many parameters, to obtain mathematical results on its stability at present (the mathematical knowledge of dynamical systems does not extend very far beyond two degrees of freedom).

To decide whether the initial conditions of the Solar System lead to a quasiperiodic or a chaotic solution, we have two approaches available: perturbation techniques and numerical integration. Improvements in computer speeds have enabled several numerical integrations on the long-term evolution of the Solar System to be made[14–19]. All of these deal with the outer Solar System (Jupiter to Pluto) and span from 500,000 years (ref. 14) to almost 10^9 years (in the latest integration[19]). One major result is numerical evidence for the chaotic motion of Pluto[19] from computation of its maximum Lyapunov exponent, which was found to be about 1/20 Myr^{-1}. This result gives the limit of predictability for the motion of Pluto, but as Pluto does not significantly perturb the rest of the Solar System, it yields no information on the limit of predictability of the essential part of the Solar System.

The direct numerical integration of the Solar System is reliable, but cannot as yet take into account the inner planets (Mercury, Venus, the Earth and Mars), for which orbital motion is too rapid. Here I use perturbation theory to remove the short periods from the motion equations; the averaged equations of the Solar System are then numerically integrated by Adam's method using a very large (500 yr) step size. I have constructed an extensive analytical differential system that gives the secular evolution of the orbits of the eight main planets of the Solar System, accurate up to second order with respect to the planetary masses and to fifth order in eccentricity and inclination. Corrections for the secular effects of the Moon and relativity are included, and a final differential system of about 150,000 polynomial terms[9] is obtained. The accuracy of the solution obtained by numerical integration of this system was checked by comparison with the complete numerical ephemeris of the Solar System DE102 (ref. 20) over the range of DE102, or 44 centuries[10]. This system was then numerically integrated over 30 Myr and its solution treated with a modified Fourier analysis to derive a solution of a quasiperiodic form over 10 Myr (ref. 21). At the same time, I found that there were many resonances in the long periods of the inner Solar System which prevented a good convergence of the solutions, suggesting that the inner Solar System is unstable[22] (similar problems of convergence have been encountered to a lesser degree by Nobili *et al.*[23] in their 100-Myr integration of the outer planets). The secular system has now been improved slightly by a small correction obtained by adjustment of three of the diagonal terms of the linear part of the secular system in order to take partially into account the third-order contribution with respect to the masses. This small change essentially improved the solution for the outer planets.

I have integrated the full system over 200 Myr. Applying my Fourier techniques to the numerical output, it was not possible to obtain a quasiperiodic solution valid over the whole timespan: when applied to different intervals of the timespan, the Fourier analysis showed significant variations of the main secular

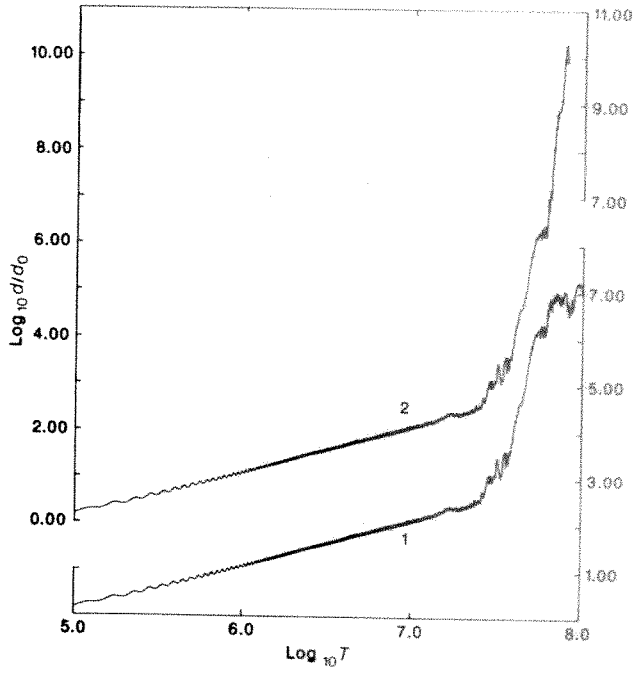


FIG. 1 Divergence of two nearby orbits. We plot $\log_{10}(d/d_0)$ versus $\log_{10}T$ (where T is time in years) over 100 Myr. At the beginning, the distance of two nearby orbits increases at a nearly linear rate. After about 30 Myr, an exponential divergence of the orbits dominates. The initial relative distance in the phase space is $d_0=10^{-7}$. In the absence of renormalization, the distance is bounded after ~60 Myr (curve 1). With a renormalization every 40 Myr, the second orbit stays close to the first and the cumulated distance is not bounded (curve 2).

frequencies of the inner planets, whereas for the outer planets, secular frequencies were steady (J. L., manuscript in preparation). All these facts suggest that the motion of the Solar System is chaotic.

The Lyapunov exponent measures the exponential rate of divergence of two nearby orbits. In quasiperiodic motion it should be zero. A non-zero Lyapunov exponent indicates chaotic motion[24,25]. The maximum Lyapunov exponent was estimated by computing the distance between two nearby orbits (with relative initial distance $d_0 = 10^{-7}$) in three different ways (Figs 1 and 2): either without renormalization (in this case the distance is bounded after ~60 Myr) or with renormalization every 40 Myr or every million years. In the three cases, I found the same Lyapunov exponent with the surprisingly high value of about $1/5\ \mathrm{Myr}^{-1}$.

This result gives a measure of the limit of predictability of my secular system: we have $d(T) \approx d(0)\, e^{T/5}$, where $d(T)$ is the distance of two close orbits in the phase space at time T in Myr. This shows that it is possible to give a precise solution of the Solar System over 10 Myr but not over 100 Myr (a perturbation as small as 10^{-10} of the initial conditions will lead to 100% discrepancy after 100 Myr). This result is particularly significant for the Milankovitch theory of palaeoclimate and the use of the orbital theory of the Earth as a timescale for changes in climate[26].

The chaotic behaviour of the Solar System comes mainly from the secular resonances among the inner planets (Mercury, Venus, the Earth and Mars). Predictions concerning the outer planets may last somewhat longer. I have not computed the maximum Lyapunov exponent of the Solar System with the inner planets excluded but, from the very regular behaviour of the solutions over 200 Myr compared with the inner-planet solutions, it is expected to be much smaller. This would explain why until now, except in the special case of Pluto, there have been no reports of non-zero Lyapunov exponents in the numerical integrations of the outer planets.

This surprisingly high Lyapunov exponent compared with the age of the Solar System needs cautious interpretation. The computation shows that the solutions of our differential system, with the initial conditions of the Solar System, are chaotic. This differential system is a close approximation to the real Solar System. We can conclude that the motion of the Solar System, and in particular the inner Solar System, is close to being chaotic, but the exact meaning of 'close' is still difficult to evaluate. Using perturbation methods, I have estimated the quality of the approximations used by comparison with the available numerical integrations[10,21]. This secular theory is more precise than any previously computed but in order to confirm these results, the computations should be repeated with a higher order of approximation. □

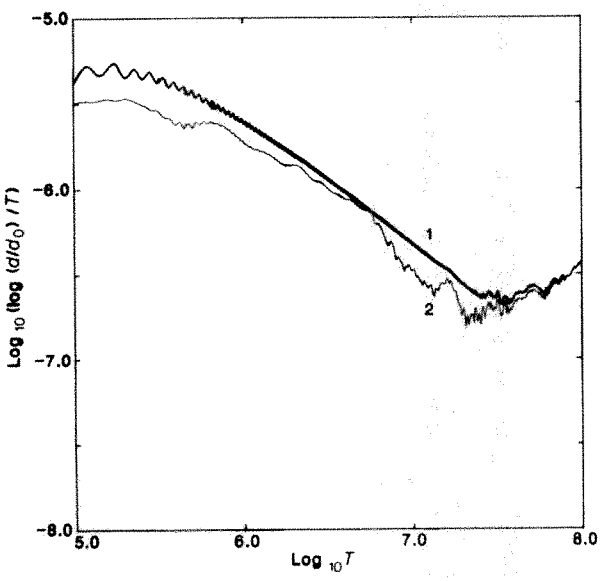


FIG. 2 Computation of the maximum Lyapounov exponent. We plot $\log_{10}(\log(d/d_0)/T)$ versus $\log_{10} T$ (where T is the time in years) over 100 Myr, with renormalization every 40 Myr (curve 1) or with renormalization every million years (curve 2) (the two curves do not coincide for the first million years because of different initial conditions). In the two cases, we obtain the same results for large values of T, which give a maximum Lyapunov exponent of about $10^{-6.7}\ \mathrm{yr}^{-1}$ ($1/5\ \mathrm{Myr}^{-1}$) for the secular system in eccentricity and inclination of the Solar System.

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The earliest known solar eclipse record redated

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AN astronomical event recorded on a clay tablet found in 1948 among the ruins of the ancient city of Ugarit, in what is now Syria, was identified 20 years ago as a description of a total solar eclipse that occurred on 3 May 1375 BC[1,2]. The dating of ancient solar eclipses provides reference points to fix the long-term evolution of angular momentum in the Earth–Moon system[3]. We have reanalysed the Ugarit eclipse record[4]. A new historical dating of the tablet, and mention in the text of the visibility of the planet Mars during the eclipse as well as the month in which it occurred enables us to show that the recorded eclipse in fact occurred on 5 March 1223 BC. This new date implies that the secular deceleration of the Earth's rotation has changed very little during the past 3,000 years.

Tablet KTU 1.78 was discovered in 1948 among the remains of the Western Palace Archive in Ugarit[5]. The contents of this archive show that it was used over the entire span of the Late Bronze Age III period (around 1350–1175 BC)[6]. However, of all the different genres found in the various archives of Ugarit, only the legal texts and treaties were kept for future reference throughout this period. The other genres were represented only

by texts dating to the last half-century of Ugarit's existence[6]. Therefore, it is likely that KTU 1.78 dates from around the second half of the thirteenth century or the first quarter of the twelfth century BC.

The text recorded on tablet KTU 1.78 was studied in detail by Sawyer and Stephenson[2], who were the first to realize that the astronomical event described may refer to a total solar eclipse. The text has recently been studied again by van Soldt[4]. The new translation runs as follows:
(Obverse) "On the . . . day of the new moon in (the month) *ḫiyaru* the Sun went down, its gate-keeper was *Ršp*". (Reverse) "Two livers were examined: danger".

The meaning of the phrase "*bit*", left open in the translation above, is problematic. Sawyer and Stephenson rejected the most obvious translation of "*it*" as "six" because a solar eclipse can occur only close to the beginning of a lunar month. Van Soldt[4] offers "the sixth hour" as an admittedly speculative possibility. As already pointed out by Sawyer and Stephenson, *Ršp* is probably to be identified with the planet Mars.

The correct translation of the text on the reverse side of the tablet is due to Dietrich *et al.*[7]. Apparently the anxiety caused by the eclipse of the Sun and the sudden appearance of Mars had to be resolved by an explanation through liver divination.

To be able to fix more precisely the date at which the eclipse was observed we need to know the place of the month *ḫiyaru* in the Ugaritic calendar. The Ugaritic calendar was a lunar one consisting of twelve months, of which ten are known by name. Making use of a collation of a key text in the Damascus Museum by one of us (W.H.v.S., unpublished work), we were able to establish the order of the months[4]. According to de Moor[8], the year in Ugarit started with the first day of *riš yn*, "the new Moon nearest to the autumn equinox". The reconstructed calendar and its approximate relation to the months in the Julian calendar in 1200 BC are summarized in Table 1. The synchronization to the Julian calendar can only be approximate because by occasional intercalation the beginning of the year was kept close (within about four weeks) to the date of the autumn equinox. We conclude that the first day of *ḫiyaru* must have fallen in the second half of February or the first half of March.

Based on the considerations above we draw up a list of conditions which an attempted dating of the solar eclipse in KTU 1.78 has to satisfy. (1) The year in which the eclipse took place should preferably be found between 1250 and 1175 BC. (2) The planet Mars must have been visible during the eclipse. (3) The date (the "day of the new Moon" in the month *ḫiyaru*) has to be set in the second half of February or in the first half of March.

Sawyer and Stephenson[2] identified four solar eclipses that could have been total in Ugarit during the period 1450–1200 BC: on 14 July 1406 BC, 3 May 1375 BC, 8 January 1340 BC and 5 March 1223 BC (all dates are given according to the Julian calendar). They selected the one that occurred on 3 May 1375 BC as the eclipse referred to in the tablet, based on the fact that it takes place in April–May, the time of year that they associated with the month of *ḫiyaru*. This association is based on an erroneous identification of *ḫiyaru* with the Babylonian month *ajjaru*.

Using a recent tabulation[9] of all solar eclipses observable in the ancient Near East from 3000 BC to AD 0, we find that only those of 1406 BC, 1375 BC and 1223 BC could have come close to being total in Ugarit. Between 1250 BC and 1175 BC the eclipse of 5 March 1223 BC is the only possible candidate.

We must now ask whether Mars was in the sky at the time of this eclipse. By using the series expansions of planetary perturbations published by van Flandern and Pulkkinen[10], we are able to calculate by computer solar, lunar and planetary positions for arbitrary epochs. All time-dependent terms in the solar, lunar and planetary elements listed in the Explanatory Supplement of the Astronomical Ephemeris[11] (ESAE) are included in these calculations. The accuracy of the resulting positions is estimated at about one arcmin for dates more recent than 2,000 years ago. For dates after 600 BC the results can be compared with the less accurate tables of Tuckerman[12]. The calculated positions usually agree to within 0.1°.

Of the three candidate eclipses mentioned above, the one occurring on 5 March 1223 BC is the only one during which the planet Mars was above the horizon. More significantly, Mars was located at 337.0° ecliptic longitude and −0.8° ecliptic latitude, only 3.5° from the centre of the eclipsed Sun at ecliptic longitude 333.6°.

The observation that Mars was present during the eclipse is a strong indication that the eclipse must indeed have been total. Muller and Stephenson[13] discuss the visibility of stars and planets during a solar eclipse and conclude that the brightest stars and planets are usually visible during a total eclipse, which is consistent with many eyewitness accounts.

Using formulae given by Allen[14], we have calculated a visual magnitudes for all planets, and all stars brighter than Mars, above the horizon during the eclipse. The results are listed in Table 2. Visual magnitudes for the stars were taken from ref. 15. The data in Table 2 show that Mars was amongst the ten brightest objects in the sky. Its proximity to the eclipsed sun may have decreased its observability because of the coronal glow, but may also have enhanced the likelihood of its observation.

The "day of the new Moon" for the eclipse date does not make sense in a Babylonian-type lunar calendar, for which months began at sunset on the day of first crescent visibility. It fits exactly[4], however, if we assume an Egyptian-type lunar calendar, for which the month began at sunrise on the day of first crescent invisibility and the second day of the month was named "new crescent day"[16]. It is not unexpected to find the Egyptian calendar in Ugarit. We know that it was used in ancient Israel[17], and Egyptian influence in Ugarit during the second millenium BC is attested in several sources[18].

TABLE 1 The order of the months in the Ugaritic Calendar and their relation to the Julian Calendar in 1200 BC

1	*rlš yn*	Sep/Oct	7	*ḫlt*	Mar/Apr
2	*nql*	Oct/Nov	8	*gn*	Apr/May
3	*mgmr*	Nov/Dec	9	*ltb*	May/Jun
4	*pgrm*	Dec/Jan	10	?	Jun/Jul
5	*lb'lt*	Jan/Feb	11	?	Jul/Aug
6	*ḫyr*	Feb/Mar	12	*lttbnm*	Aug/Sep

TABLE 2 The brightest stars and planets above the horizon in Ugarit on 5 March 1223 BC at 13^h20^m Local Mean Time, listed in order of decreasing visual brightness

Star	Name	Az* (degrees)	El*	m_v (magnitude)
	Sun/Moon	337	41	
	Venus	22	61	−4.58
α CMa	Sirius	65	2	−1.46
α Lyr	Vega	231	15	0.03
α Aur	Capella	99	63	0.08
β Ori	Rigel	49	20	0.12
α CMi	Procyon	89	13	0.38
α Ori	Betelgeuse	67	29	0.50
	Mercurius	317	20	0.67
α Tau	Aldebaran	51	47	0.85
β Gem	Pollux	105	29	1.14
α Cyg	Deneb	246	35	1.25
	Mars	341	43	1.28

* Positions are given in azimuth and elevation rounded off to full degrees. Azimuth is measured anticlockwise along the horizon from the south (Az = 0°), elevation is measured perpendicular to the horizon (zenith corresponds to El = 90°).

If time was also kept in the Egyptian way (ten day-hours from sunrise to sunset[19]) the suggested translation of *btt* as "sixth hour" gains credibility because the eclipse took place at 13^h20^m, whereas sunrise and sunset occurred at 6^h45^m and 17^h45^m Local Mean Time, respectively.

Using the order of the months in Table 1 we find that the beginning of the Ugaritic year, the first day of the month *riš yn*, fell on 7 October 1224 BC, quite close to the actual equinox date of 1 October.

We have investigated the implications of the new eclipse date for the secular deceleration of the Earth's rotation rate. We tested our calculations by comparison with modern data of a number of twentieth-century eclipses[20].

To include the effect of the secular accelerations of the Earth and Moon for dates in the past, the ESAE recommends the following expressions for the secular increment in the mean longitude of the Moon, λ_m, and for the difference between Universal and Ephemeris Times:

$$\Delta\lambda_m = -11.22\ T^2 \text{ arcsec} \quad (1)$$

$$\Delta T = \text{ET} - \text{UT} = 24.3 + 72.3\ T + 30.0\ T^2 \quad \text{seconds} \quad (2)$$

where T is the time in Julian centuries since 1.0 January 1900. The magnitude of $\Delta\lambda_m$ corresponds to an acceleration rate of the Moon's mean longitude of $\dot{n} = -22.44$ arcsec century^{-2}. According to equations (1) and (2), in 1223 BC the effect of the secular accelerations produces large differences in lunar longitude and in time, 3.0° and 8.8 hours respectively.

The standard ESAE value of the lunar acceleration rate is confirmed by recent studies. The McDonald Observatory lunar laser-ranging experiment[21] gives a value of $\dot{n} = -23.8 \pm 1.5$ arcsec century^{-2}, equal, within errors, to the standard value. A re-analysis[22] of modern (1717–1973) lunar and planetary observations yields $\dot{n} = -22.2 \pm 0.8$ arcsec century^{-2}, which is fully consistent with the lunar laser-ranging result and the standard value.

To accommodate variations in the deceleration rate of the Earth relative to the standard ESAE value we add to ΔT in equation (2) a time parameter δt. If the adopted deceleration rate is too large or too small, δt will systematically decrease or increase going further into the past.

We have analysed the eclipse of 5 March 1223 BC in Ugarit (geographical coordinates 35°37′ N, 35°47′ E) by calculating its magnitude as a function of time for several values of the parameter δt. Requiring that the eclipse must have been total results in the condition $-3^m \leq \delta t \leq 6^m$. The accuracy of this result is estimated to be about 3 min. Thus the average deceleration of the Earth's rotation rate over the past 3,000 years is well described by the standard ESAE value of the deceleration parameter, and long-term variations (on the scale of centuries) in the deceleration rate over this period have probably been small. This result is of geophysical importance because it helps to establish the nature of the processes, other than the lunar tidal torque[23], affecting the rotation rate of the Earth. □

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Superconductivity at 27 K in fluorine-doped Nd_2CuO_4

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THE recent report[1] of superconductivity in $Nd_{1.85}Ce_{0.15}CuO_4$ is the first example of a high-transition-temperature (high-T_c) superconductor with electrons as the charge carriers and a formal copper oxidation state of less than +2. All previously characterized copper oxide superconductors are hole-doped, and the copper is formally oxidized. Here we show that substitution of fluorine for oxygen in T′-phase Nd_2CuO_4 provides an alternative route to achieving formal reduction of copper, electron conductivity and superconductivity at temperatures as high as 27 K. This result is unusual, because anionic rather than cationic substitution gives rise to the superconductivity.

We explored several routes to the preparation of $Nd_2CuO_{4-x}F_x$; the best results were obtained by heating stoichiometric mixtures of CuO, Nd_2O_3 and freshly prepared NdF_3 in air at 900 °C for 14 h followed by pelletizing and heating at 890 °C for 14 h in flowing N_2. A single phase with the T′ structure was obtained for samples of nominal composition $Nd_2CuO_{4-x}F_x$ with $0 < x < 0.4$. Fluorine analysis was carried out by the Schwarzkopf Microanalytical Laboratory on a sample of nominal composition $x = 0.4$; the analysed composition was found to be $x = 0.28$. (Hereafter we will refer always to nominal compositions.) Loss of fluorine could occur in the initial firing step by hydrolysis of NdF_3 to NdOF (ref. 2). After the initial firing in air, X-ray powder-diffraction patterns of the $Nd_2CuO_{4-x}F_x$ samples showed the characteristic lines of the T′ structure, with small amounts of NdOF; the NdOF lines disappeared after firing in N_2. Lattice parameter refinements showed that fluorine substitution results in a small contraction (up to 0.2%) of the c axis and a small expansion (up to 0.3%) of the a and b axes; for example, the lattice parameters of the $Nd_2CuO_{3.6}F_{0.4}$ sample were found to be $a = 3.961 \pm 0.006$ Å,

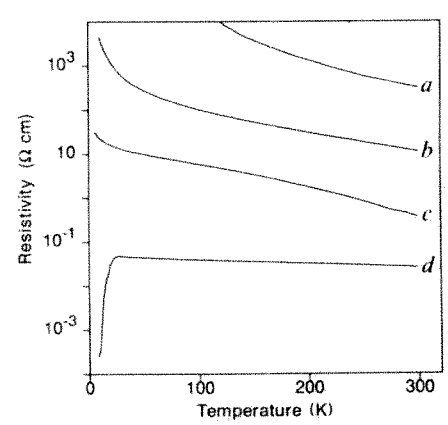


FIG. 1 Electrical resistivity of *a*, Nd_2CuO_4 prepared in air at 900 °C; *b*, Same sample after annealing in N_2 at 890 °C for 14 h; *c*, Sample of nominal composition $Nd_2CuO_{3.6}F_{0.4}$ prepared in air at 900 °C; *d*, Same fluorinated sample after annealing in N_2 at 890 °C for 14 h.

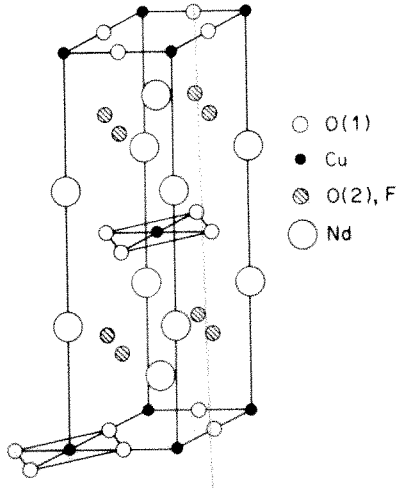


FIG. 2 The T'-Nd_2CuO_4 structure, showing the suggested location of the fluorine atoms in $Nd_2CuO_{4-x}F_x$.

$c = 12.13 \pm 0.02$ Å. The average Cu oxidation state in samples of $Nd_2CuO_{4-x}F_x$ was determined by iodometric titration[3]. The unsubstituted material Nd_2CuO_4 was found to have a Cu oxidation state of $+2.00 \pm 0.02$ both before and after firing at 890 °C in nitrogen. The $x = 0.4$ sample gave a Cu oxidation state of $+1.89 \pm 0.02$ after the initial firing in air and $+1.76 \pm 0.02$ after firing in N_2 overnight. Combining the fluorine and oxidation-state analyses gives an overall composition of $Nd_2CuO_{3.74}F_{0.28}$, which is very close to the stoichiometric limit of four anions per Cu. These results indicate that nitrogen annealing at 890 °C results in a greater degree of fluorine substitution in $Nd_2CuO_{4-x}F_x$ compared with air firing, without changing the total anion stoichiometry.

Substitution of F for O was also attempted in the other rare-earth copper oxides M_2CuO_4 (where M represents Pr, Sm, Eu or Gd), all of which have the T' structure. Single-phase materials of nominal composition $Pr_2CuO_{4-x}F_x$ $(0 < x < 0.4)$ could be prepared by a method similar to that described above. Pure Sm, Eu and Gd analogues, however, could not be prepared because of increasingly facile reductive decomposition under N_2 to mixtures of the corresponding rare-earth oxides, oxyfluorides and Cu_2O. A similar reductive decomposition under N_2 was observed for $Pr_2CuO_{4-x}F_x$ and $Nd_2CuO_{4-x}F_x$ at temperatures above 950 °C and 900 °C respectively. Recent studies have shown that the cerium-substituted superconductor $Nd_{1.85}Ce_{0.15}CuO_4$ also decomposes at elevated temperature under N_2.

Figure 1 shows resistivity as a function of temperature for $Nd_2CuO_{4-x}F_x$ (nominal composition $x = 0$, 0.4). The sample of $Nd_2CuO_{3.6}F_{0.4}$ fired in air shows a slope that is characteristic of a semiconductor, with conductivity substantially higher than that of Nd_2CuO_4, but no indication of superconductivity. After firing in N_2 the slope is reduced and there is a clear superconducting transition. The onset temperature for superconductivity was found to increase with nominal fluorine content to a maximum of 27 K for $x = 0.3$, and then to decrease, reaching 10 K in an $x = 0.5$ sample, which was no longer single-phase. No superconductivity was observed in a sample of nominal composition $x = 0.1$. The superconducting transitions were broad in all cases and depended on the details of the sample composition and preparation. The sharpest transition observed was in a sample of $Nd_2CuO_{3.6}F_{0.4}$; in this case the superconducting onset temperature was 21 K and zero resistance was observed at 12 K. Flux-exclusion measurements on two samples with $x = 0.2$ and 0.4 each showed 13% Meissner effects. All of the fluorinated samples exhibited n-type conductivity at room temperature, as determined by Seebeck-effect measurements. The fluorinated Pr copper oxides $Pr_2CuO_{4-x}F_x$ $(x > 0.2)$ also superconduct; the a.c. susceptibility exhibits a strong diamagnetic signal at 4.2 K and the resistivity shows a sharp drop at temperatures as high as 13 K (for the $x = 0.4$ sample). However, zero resistance at 4.2 K was not achieved in any of the Pr samples. Although samples containing rare earths other than Pr could not be made as a single phase for the reasons mentioned above, screening of multiphase materials by a.c. susceptibility has failed to show superconductivity.

Although F and O are similar in size, their mutual substitution is often limited, especially in transition-metal compounds. The extent of substitution of fluorine in $YBa_2Cu_3O_7$ is limited, and its effect on superconductivity is detrimental[4], because $YBa_2Cu_3O_7$ is a p-type conductor in the normal state so that fluorine doping reduces the carrier density. That substantial fluorine substitution is possible in $Nd_2CuO_{4-x}F_x$ may be explained on the basis of its T' structure[5] (Fig. 2). The structure contains two oxygen atom sites: O(1) is bonded to Cu, forming the Cu–O square net common to all copper oxide superconductors, whereas O(2) is bonded to Nd, forming a fluorite-type layer between the CuO_2 sheets. It is the O(2) atoms that we believe to be substituted by F. Substitution on this site would not disrupt the CuO_2 plane and would make the bonding of the $NdO_{2-x}F_x$ layer similar to that of NdOF, which has a distorted fluorite structure.

The phase purity and substantial flux exclusion below T_c of the T'-phase $Nd_2CuO_{4-x}F_x$ indicate bulk superconductivity, but the broad superconducting transitions and semiconducting slope of the electrical conductivity above T_c suggest that the samples are not completely homogeneous. Similar behaviour has been observed in ceramic samples of other solid-solution superconductors $La_{1.85}Sr_{0.15}CuO_4$ (ref. 6) and $Pb_2Sr_2Y_{1-x}Ca_xCu_3O_8$ (ref. 7). □

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Optical study of the metal–insulator transition on $Ba_{1-x}K_xBiO_3$ thin films

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THE superconducting perovskite (Ba, K)BiO_3 (refs 1, 2) has the highest transition temperature known ($T_c \approx 31$ K) aside from the copper oxide compounds[3]. This system becomes superconducting upon the substitution of monovalent potassium for divalent barium in the parent insulator $BaBiO_3$, analogous to the substitution of strontium for lanthanum in La_2CuO_4. To understand whether the mechanism of the superconductivity is the same as in the copper oxide compounds, it is important to know the origin of the insulating state of the parent material, as well as the basic character of the metallic state induced by the cation substitution. In the case of its predecessor Ba(Bi,Pb)O_3, optical measurements were used to investigate the change in electronic state across the metal–insulator transition at 65% lead doping[4]. Here we report the successful synthesis of (Ba,K)BiO_3 thin films, which has allowed

us to measure the optical spectrum, as well as the transport coefficients of (Ba,K)BiO_3. Our results, combined with the previous report of the X-ray crystal structure[5], strongly suggest that the Peierls gap, responsible for the insulating state of $BaBiO_3$, almost completely vanishes at the metal–insulator transition, resulting in a metallic state described by band theory[6].

The $Ba_{1-x}K_xBiO_3$ thin films were epitaxially grown on $SrTiO_3$ (110) substrates using an r.f.-magnetron sputtering method with substrate temperature of 400 °C. The sputtering targets were prepared from a powder mixture of Bi_2O_3, BaO and KO_2 by means of the two-step synthesis procedure[7]. The composition of the targets was adjusted so as to reduce the deviation of the composition of the sputtered films from the stoichiometric ratios, because films sputtered from a stoichiometric target contained a large number of K and Bi vacancies. The chemical composition of the films was determined roughly by electron-probe microanalysis and X-ray fluorescence analysis. In particular, the K composition (x) was estimated from the lattice constant[8]. The K composition was sensitive to both the argon gas pressure and the oxygen partial pressure. The superconducting films with the highest potassium concentration, $x = 0.39$, were obtained by using a gas mixture of argon with 1% oxygen at a pressure of $\sim 10^{-2}$ torr. Under these conditions, the deposition rate was ~ 2 Å s^{-1} and the typical thickness of the films was ~3,000 Å. Because of the reducing growth conditions, the films had large numbers of oxygen vacancies; these were filled by annealing the films in flowing oxygen at ~500 °C for 1 h.

Figure 1 shows the X-ray diffraction pattern; the films are almost perfectly orientated in the (110) direction without any trace of a secondary phase. The width of the diffraction peak is comparable to that of the single-crystalline substrate, which demonstrates the good quality of the films.

Not all of the films were superconducting. After oxygen annealing, the films with $x > 0.35$ had a very sharp superconducting transition, whereas films with $x < 0.35$ remained semiconducting. The superconducting transition was observed with both resistivity and magnetization measurements. As seen from the inset of Fig. 2, the onset transition temperature is 23 K, the transition width being no more than 0.2 K. The sharp transition and the high critical-current density at 4.2 K of 7×10^4 A cm^{-2} (which is comparable with the value of 2.5×10^5 A cm^2 reported for a Ba(Bi,Pb)O_3 single-crystalline thin film[9]) testify to the homogeneity of the films.

From 300 to 150 K, the resistivity is almost constant and is <1 mΩ cm, indicating that grain boundaries have a smaller effect than in the polycrystalline specimens, for which significantly higher resistivity has been reported[10,11]. Below 150 K the resistivity has a positive (that is, metallic) temperature coefficient which has never been observed for polycrystalline samples.

The metallic nature is also confirmed by the optical reflectivity spectrum. Figure 2 shows a reflectivity edge due to free-carrier plasma for $Ba_{0.63}K_{0.37}BiO_3$ thin films. This reflectivity spectrum fits the Drude free-electron model well in the energy range from 0.5 to 2.0 eV. The fit is not so good in the energy region above 2 eV because of the presence of an absorption band at ~3 eV. The fitting parameters are $\hbar\omega_p = 1.59$ eV, $\hbar\gamma = 0.92$ eV and $\varepsilon_\infty = 3$, where ω_p, γ and ε_∞ are the plasma frequency, the damping factor and the optical dielectric constant, respectively. The 'bare' plasma energy $\varepsilon_\infty^{1/2}\hbar\omega_p$ (formally equal to $(4\pi ne^2/m^*)^{1/2}$, m^* and n being the effective mass and the density of carriers, respectively) is 2.8 eV, which is ~50% smaller than the value of 4.1 eV given by band calculations[12]. However, in view of the uncertainty in the value of ε_∞ due to the absence of higher-energy reflectivity data, the agreement between experimental results and band theory calculations is satisfactory. The estimated conductivity at zero energy, $\sigma_{d.c.} = \varepsilon_\infty\omega_p^2/\gamma$, obtained from these parameters is $\sim 2 \times 10^3\ \Omega^{-1}\ cm^{-1}$ and is nearly consistent with the resistivity measurement.

The spectrum is significantly different from that of superconducting $BaBi_{0.25}Pb_{0.75}O_3$, for which $T_c = 12$ K. In the latter case, the plasma spectrum does not fit the Drude model even in the energy region below 1 eV owing to the specific circumstances described below[4].

Taking advantage of the synthesis of these thin films, we investigated the metal–insulator transition using transmittance measurements. Figure 3*a* shows the optical-conductivity spectra obtained from both reflectivity and transmission measurements on $BaBiO_3$ films and those with K substituted at $x = 0.17$, 0.28 and 0.37. For comparison, Fig. 3*b* shows the optical-conductivity spectra of $BaBi_{1-x}Pb_xO_3$, which were obtained from the reflectivity spectra by means of a Kramers–Kronig transformation[4].

A rather sharp dominant absorption peak is observed at 2 eV in the spectrum of $BaBiO_3$, the parent compound of both systems, which we therefore interpreted to be a Peierls insulator with an optical energy gap of ~2 eV (ref. 4), similar to the simple cubic metals originally considered by Peierls[13]. In $BaBiO_3$, the

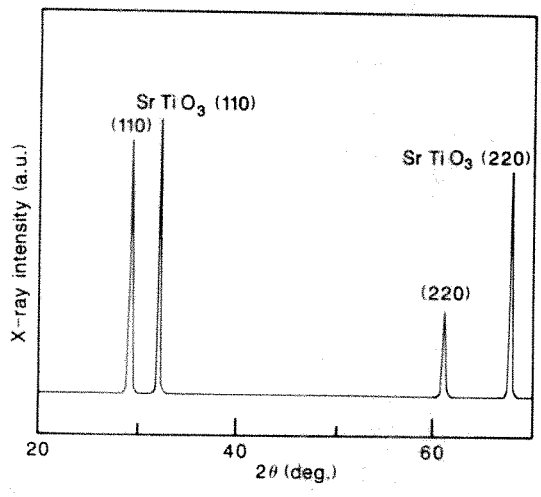


FIG. 1 Cu $K\alpha_1$ X-ray diffraction pattern of a 3,000-Å-thick film of $Ba_{1-x}K_xBiO_3$ on a $SrTiO_3$ (110) substrate. The calculated lattice parameter was 4.275 Å, which corresponds to a K concentration $x = 0.39$, according to ref. 8. X-ray intensity is expressed in arbitrary units.

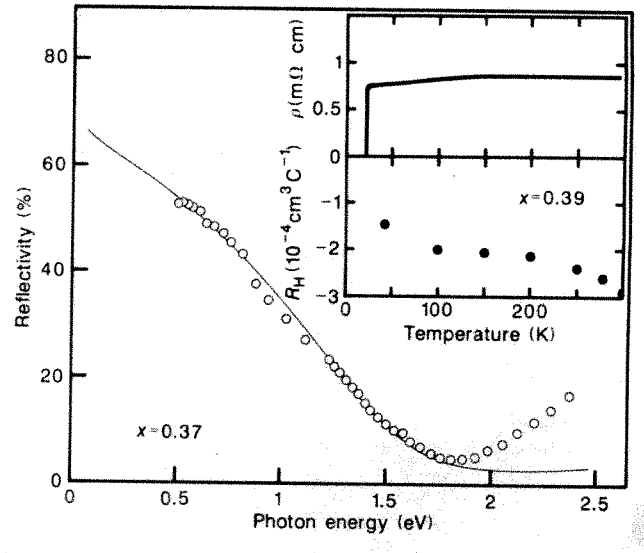


FIG. 2 The reflectivity spectrum for a 1,000-Å-thick film with $x = 0.37$ in the energy region from 0.5 to 2.4 eV. The solid line shows the spectrum calculated based on the Drude model with the plasma frequency $\hbar\omega_p = 1.59$ eV, the damping frequency $\hbar\gamma = 0.92$ eV and the optical dielectric constant $\varepsilon_\infty = 3$. The inset shows the temperature dependence of the electrical resistivity, ρ, and the Hall coefficient, R_H, of a thin film with $x = 0.39$ and a thickness of 3,000 Å.

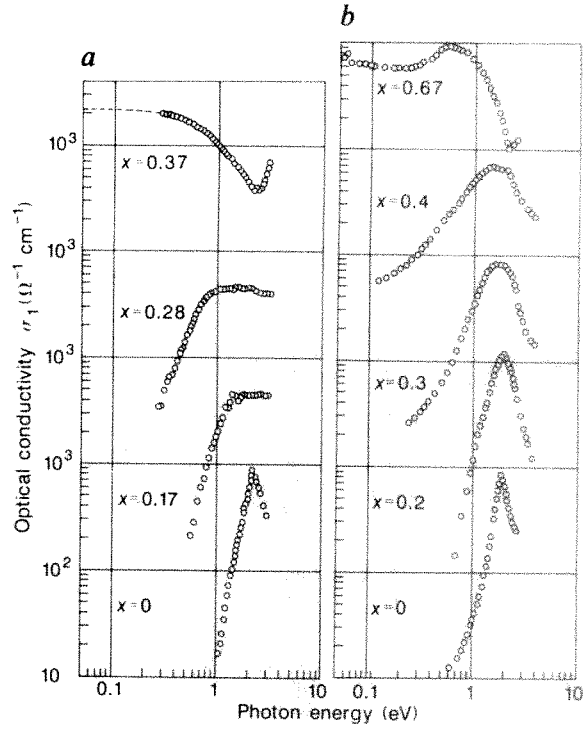


FIG. 3 *a*, Optical-conductivity spectra for $Ba_{1-x}K_xBiO_3$ thin films with $x = 0$ ($BaBiO_3$), 0.17, 0.28 and 0.37. The thickness of the films were 3,000, 20,000, 10,000 and 1,000 Å, respectively. The broken line in the spectrum for the film with $x = 0.37$ denotes the calculated spectrum below 0.25 eV using the Drude model with the parameters stated in the text. *b*, Optical-conductivity spectra for the $BaBi_{1-x}Pb_xO_3$ system obtained from the reflectivity spectra by means of a Kramers–Kronig transformation.

Bi atoms occupy the sites of a simple cubic lattice, but the oxygen octahedra surrounding Bi sites are alternately expanded and compressed (breathing-mode distortion), as observed in neutron-scattering experiments[14]. Such periodic lattice distortion leads to Bi^{3+}–Bi^{5+} charge ordering or charge disproportionation ($Bi^{4+} + Bi^{4+} \rightarrow Bi^{3+} + Bi^{5+}$), giving rise to so-called charge density waves (CDWs). As a consequence of CDW formation, an energy gap (Peierls gap) opens at the centre of the conduction band composed of Bi(6*s*)–O(2*p*) antibonding orbitals[15].

When Bi is partially substituted by Pb, the absorption peak is gradually broadened and shifts towards a lower energy with a tail extending to much lower energies. This has been attributed[16] to the formation of Pb states within the Peierls gap of $BaBiO_3$. The metal–insulator transition is considered to take place at 65% Pb substitution, when the Pb(6*s*) states fill up the energy gap. In this picture, the Peierls gap persists as a 'pseudogap' even in the metallic phase[4]. This is supported by the fact that the optical-conductivity spectrum for the metallic compound $BaBi_{0.33}Pb_{0.67}O_3$ retains the absorption peak.

The case of K doping is somewhat different. As in the case of Pb substitution, the absorption edge shifts towards lower energy with increasing K, clearly demonstrating a decrease in the optical energy gap. However, the optical conductivity still rises steeply without any appreciable low-energy tail and the edge shifts faster than in Pb-substituted compounds. Single-crystal X-ray analysis for compounds with little K substitution[5] shows two distinct Bi sites generated by a kind of breathing-mode lattice distortion. The optical spectrum suggests that the K-substituted compound is still a Peierls insulator but that the energy gap is reduced more rapidly by K-substitution. This is consistent with the fact that, in the $Ba_{1-x}K_xBiO_3$ system, the critical composition at which the metal–insulator transition takes place is $x = 0.35$, whereas it does so for $x = 0.65$ in the $BaBi_{1-x}Pb_xO_3$ system. The difference between the two systems arises from the difference in the electrostatic potential produced by the different substitutions in the parent compound $BaBiO_3$. It is inferred from the optical spectra that substituted Pb atoms give rise to energy states within the energy gap whereas K atoms do not.

The optical conductivity spectrum of the metallic compound with $x = 0.37$ is featureless below the plasma energy, suggesting that the energy gap would vanish almost completely at the insulator–metal transition, in contrast to the case of $Ba(Bi,Pb)O_3$ where the gap remains even in the metallic phase. This might be the reason for a lower T_c value ($T_c = 12$ K) in this compound.

Measurements of the Hall and Seebeck effects on metallic thin films give negative coefficients. The Hall coefficient, shown in the inset of Fig. 2, is -3.0×10^{-4} cm^3 C^{-1} at 300 K and the Seebeck coefficient is $-(1\text{–}2)$ μV K^{-1} in the temperature range 80–350 K. That the charge carriers are electrons is plausible if the energy gap vanishes and the resultant single conduction band is less than half-full as a result of K doping. Single-crystal X-ray diffraction[5] indicates that Bi sites remain equivalent, so there is no lattice distortion in the superconducting phase of $Ba_{1-x}K_xBiO_3$. In this respect the normal state of metallic $Ba_{1-x}K_xBiO_3$ seems completely different from that in copper oxide superconductors, in which the energy gap generated by the large Coulomb repulsive energy is preserved and the substitution of divalent for trivalent elements, for example Sr for La in La_2CuO_4, produces a hole in the lower split-off band.

The optical measurements on thin films of the $Ba_{1-x}K_xBiO_3$ system strongly suggest that K doping reduces the Peierls condensation energy in $BaBiO_3$ much more effectively than does Pb doping, and the metal–insulator transition in this system is directly related to the destruction of the Peierls gap, whereas in the $BaBi_{1-x}Pb_xO_3$ system the transition is driven by the growth of the Pb states within the Peierls gap. □

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Changes of tropical sea–air interaction processes over a 30-year period

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RECENT investigations[1–5] including radiosonde data from Southern India[6] have shown that the average tropospheric temperature in the tropics has increased since 1965 by nearly 1 °C. More importantly, the water vapour content of the middle troposphere has increased, above the equatorial Pacific, by 20–30%[4,5]. Here we report investigations on sea–air interaction processes along the main shipping routes of equatorial oceans—the only areas where there have been enough observations (Table 1) to provide a representative time series. The period 1949–79 was selected because of the general lack of bias in the air–sea parameters with the exception of wind speed. The results indicate an intensification of the hydrological cycle, especially over the warmest oceans and they also suggest tropical circulation changes.

The equatorial belt (10° S–10° N) covers an area of 88×10^6 km^2, with 77% being ocean. Along 14 sections of the busiest shipping routes (Fig. 1), about 2.4 million observations were selected from the trimmed COADS data[7], covering an area of nearly 8×10^6 km^2. We also intended to check the spurious increase[8] of wind speed. We evaluated the sea surface temperature (SST); the sea–air temperature difference (SST − AT); the saturation deficit ($q_s - q_a$, where q is the specific humidity at the sea surface, subscript s denotes saturation at the sea surface, subscript a, saturation in the air), and the scalar wind speed, v_{scal}. Preliminary results[9] indicate high spatial variability of all sea–air parameters. This was true even on adjacent sections crossing the Equator where all observations had been made on board the same ships with the same equipment (Table 1) which suggests that the wind speed data show real changes as well as the effect of some biases[8]. The saturation deficit ($q_s - q_a$) increases nearly everywhere, with varying intensity; similarly the sea–air temperature difference increases but with regional and seasonal variations. The cross-equatorial changes are particularly large along routes 3–4 and 9–10, where the southern

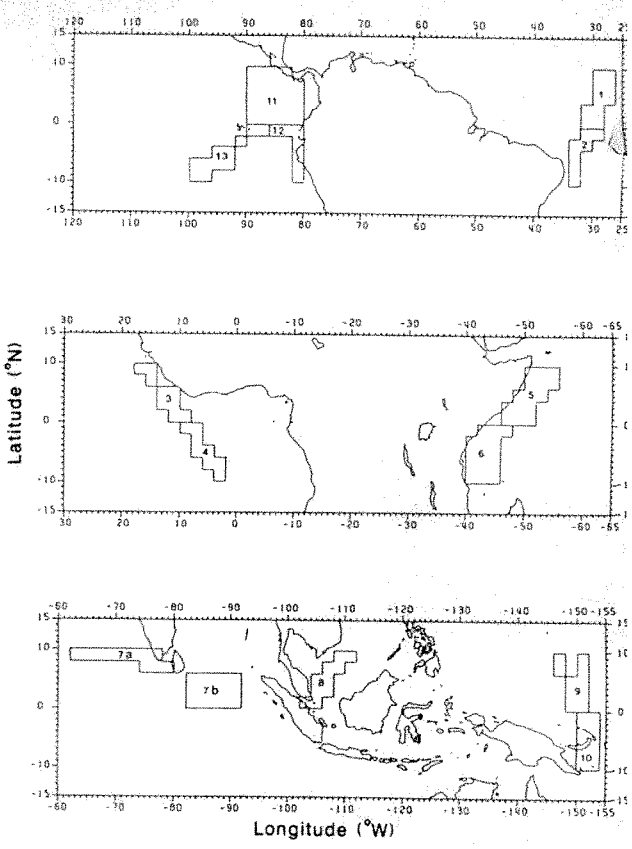


FIG. 1 Map of the selected 14 sections of ship routes in the equatorial belt

sections are affected by seasonal upwelling.

Table 2 gives 31-yr averages and trends from three region groups: the Atlantic (sections 1–4); the warmest oceans (60° 180° E, sections 7*a*, *b*–10); and the eastern Pacific (11–13). Th zonal average (14 sections) is not fully representative, becaus many routes are in areas with cool water upwelling. For com parison, SST averages from our sections are supplemented b true averages (SST*), using all 4×4° COADS fields (J. C Fletcher, personal communication). The most interesting result are from the warmest oceans on both flanks of Indonesia. I 11 of 14 sections the SST − AT trend is positive, with 7 significar

TABLE 1 Comparison of trends of sea–air parameters (unit per 31 yr) along cross-equatorial section pairs

	Atlantic				Pacific	
Sections	1	2	3	4	9*	10*
SST (°C) Year	(+0.07)	+0.46	(+0.15)	(+0.32)	+0.87	(+0.16)
DJFM†	(+0.04)	+0.41	(+0.01)	(+0.27)	+1.07	(+0.44)
JJAS‡	(+0.13)	+0.45	(+0.30)	(+0.38)	+0.80	(+0.27)
SST − AT (°C) Year	(+0.10)	+0.24	(+0.03)	+0.16	(+0.18)	(−0.05)
DJFM†	+0.13	+0.34	(+0.00)	(+0.08)	(+0.28)	(+0.24)
JJAS‡	(+0.11)	(+0.16)	(+0.03)	+0.24	(+0.01)	(−0.10)
$q_s - q_a$ (g kg^{-1}) Year	(+0.19)	(+0.21)	(+0.13)	(+0.29)	(+1.02)	(+0.16)
DJFM†	(+0.17)	(+0.20)	(+0.01)	(+0.17)	(+1.26)	(+0.80)
JJAS‡	(+0.27)	+0.28	(+0.14)	(+0.28)	(+0.84)	(−0.17)
v_{scal} (m s^{-1}) Year	+0.57	(+0.15)	+0.27	+0.63	+1.19	+1.31
DJFM	+0.69	(+0.27)	(+0.11)	+0.45	(+0.83)	(+0.66)
JJAS‡	(+0.25)	(+0.13)	+0.34	0.82	+1.21	+1.87
Number of observations per month	470	359	769	604	275	258

*Observed 1956–1979 only, corrected to 31 yr.
† DJFM, December January February March.
‡ JJAS, June July August September.

= Significance >99.9%.
– Significance >99%.
( ) Significance <95%.

the 95% level. The saturation-deficit trend is significantly positive in sections 6–9; here the 31-yr trend amounts to 0.72 g kg^{-1} or +14%. Including wind speed, these values (Table 2) are physically coherent; the strongest positive trends of SST and ($q_s - q_a$) (Fig. 2) are observed in the warmest areas. These parameters indicate a destabilization of the marine boundary layer, which cannot be systematic error (for instance, the increasing size of ships). The individual points on Fig. 2 suggest that the trends are not affected by the frequency of cold or warm El Niño–Southern Oscillation (ENSO) events.

Recent satellite measurements[10] show high correlations between ($q_s - q_a$) and evaporation (E); and weaker correlations between v_{scal} and E. This coincides with the increasing moisture in the midtroposphere (500–700 hPa, 1 hPa = 1 mbar) above the Pacific, as discussed in refs 4, 5. Here precipitable water has increased at four stations (each significant at 95%), by up to 30% from 1965 to 1984.

The simultaneous increase of SST and atmospheric moisture can be understood following recent investigations[11,12] which have revealed a nonlinear increase in the heights of convective

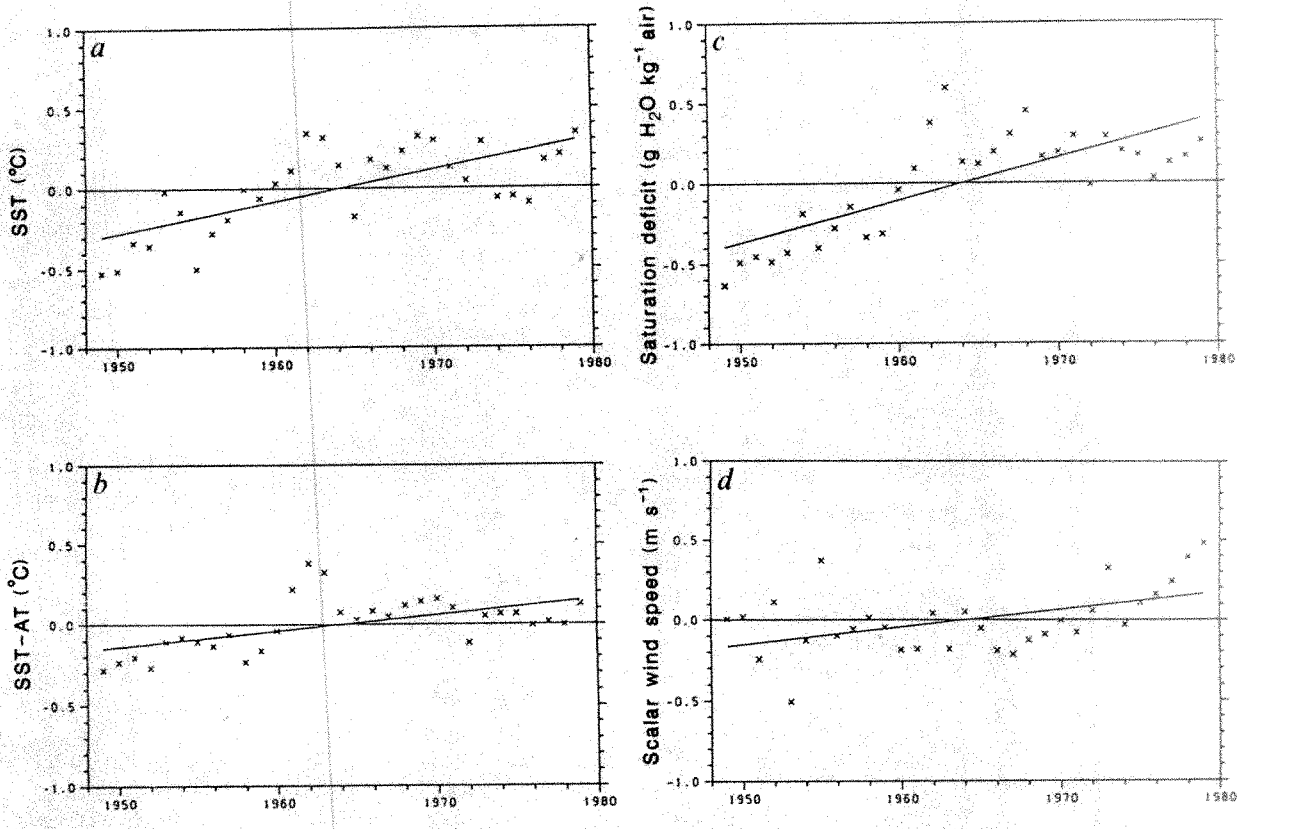


FIG. 2 Time series of selected parameters at the sea–air interface, averaged for sections 7a, 7b, 8–10 (warmest oceans) with linear annual trend 1949–79. *a*, SST; *b*, SST − AT; *c*, saturation deficit $q_s - q_a$; *d*, scalar wind speed, V_{scal}

TABLE 2 Annual averages for equatorial oceans and Indian Ocean seasonal variation

Sections		SST (°C)	SST − AT (°C)	$q_s - q_a$ (g kg^{-1})	V_{scat} (m s^{-1})	SST* (°C)
Annual averages for equatorial oceans (10° N–10° S)						
Atlantic (Sections 1–4)	M	26.58	0.48	4.72	5.07	26.79
	T_{31}	(+0.25)	+0.13	(+0.20)	+0.41	
East Pacific (Sections 11–13)	M	24.36	0.60	3.70	4.84	26.28
	T_{31}	(+0.14)	−0.13	(−0.02)	+0.52	
Warmest Oceans (Sections 7a, 7b–10)	M	28.55	0.58	5.50	4.85	28.50
	T_{31}	+0.39	+0.16	+0.55	+0.74	
Zonal averages (14 Sections)	M	26.87	0.50	4.76	5.10	27.39
	T_{31}	+0.34	+0.12	+0.33	+0.53	
Seasonal variation in the equatorial Indian Ocean (0–10° N, sections 5, 7a+7b)						
Summer (JJAS)	M	27.21	+0.02	4.00	7.73	
	T_{31}	+0.46	+0.31	+0.57	(−0.01)	
Winter (DJFM)	M	27.69	0.40	5.51	5.00	
	T_{31}	+0.45	+0.22	+0.37	+0.41	

SST*, 'true' average from complete data (4° × 4° fields, COADS). M, annual averages for the period 1949–79. T_{31}, Trend (unit = M). Significance symbols as in Table 1.

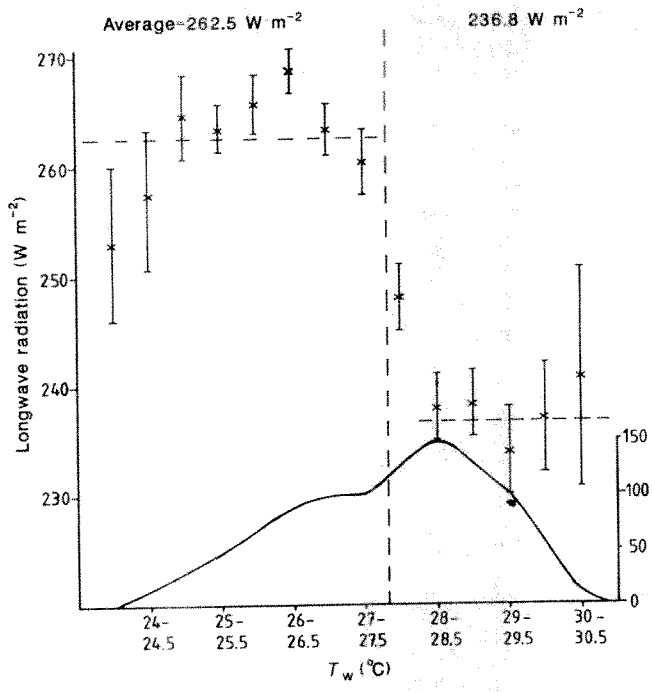


FIG. 3 Outgoing longwave radiation—representing cloud-top temperature and height—as a function of SST (T_w) at the surface of the equatorial Pacific Ocean[11]. Lower curve denotes frequency of cases.

cloud tops with increases in SST. The statistics (Fig. 3) show a jump between high, cold and low, warm cloud tops near SST = 27.5 °C. Strong convective activity driven by low-level convergent winds[12] transports water vapour to the upper troposphere, where latent heat is released by condensation. An evaluation from COADS-based data yields an ocean area of $\sim 65 \times 10^6$ km^2 with SST > 27.5 °C. With a warming of 0.39 °C over 31 years (Table 2) this area has grown, over this period, by $\sim 13 \times 10^6$ km^2 or 20%. Such figures seem to hold the key for understanding such rapid development even assuming no variation of the wind field.

These changes of sea–air parameters are correlated with an increase in E. Disregarding absolute values of E, we may estimate, on the basis of the well known bulk formula[13] the relative changes between 1949 and 1979. Assuming no change of wind speed (which is unlikely), we obtain from the $(q_s - q_a)$ trend alone an increase of E over the 'warmest' oceans of ~10%; if we allow an increase in v_{scal} of 0.25 m s^{-1}, the rise in E amounts to ~15% in 31 years. As the 1927 Beaufort wind scale used in COADS data has to be shifted upward (by 1.4–2.0 m s^{-1} after comparison with measurements in different latitudes[13–15], it is necessary to add a correction of ~+1.5 m s^{-1} to the average COADS wind speeds. Then the additional energy input from release of latent heat into the atmosphere is 10–15 W m^{-2}. This should be compared with a global value of 2–2.5 W m^{-2} from the increase in CO_2 and other greenhouse gases[16], not accounting for feedback mechanisms, since the beginning of this century. Similar ratios have been estimated before[17–19].

Independently two recent climate models have indicated a heating maximum in the tropics[19,20]. Recent model studies have also found that positive feedback coefficients operating by means of water vapour and clouds (primarily in the tropics) are distinctly higher than the snow-albedo feedback[21].

This recently discovered powerful heating source in the equatorial belt is concentrated over the eastern Indian Ocean and Western Pacific. Here the upper mixed layer of the ocean is effectively isolated from exchange with the cool layers below, owing to the great depth of the mixing layer and the lack of effective upwelling. The rise of SST, of $(q_s - q_a)$ and of E together with the increase in tropospheric temperature and moisture[4] are consistent with a strengthening of the subtropical 700-hPa westerlies in the Pacific section[22]. This suggests an intensification of the Hadley circulation in that section and has been confirmed by our investigations at 200- and 500-hPa levels (to be reported elsewhere).

If we consider the zonal SST differences between the warmest oceans and sections 11–13 as representing the Walker circulation, our data (Table 2, upper panel) show an increase of this SST difference of 6%; no radiosonde time series are available at the Equator. Land data indicate, for 1950–80, no significant trend[23] at latitudes 35–60° N. In the Indian Ocean, therefore, the rise of SST and of the heating rate (indicated by SST − AT) at sections 5, 7a and 7b (Table 2, lower panel) suggest a decrease (increase) of the meridional land–sea difference during summer (winter) that drives both monsoons in these sections. Taking into account (as above) an estimated real increase of surface wind speed +0.25 m s^{-1}, the wind data reveal a weakening of the summer monsoon and an intensification of the winter monsoon.

It seems that the warming of the tropical troposphere can be associated with more frequent deep convection correlated with SST > 27.5 °C releasing more latent heat into the atmosphere. The physical role of water vapour is not only due to its (radiative) greenhouse effect, but even more to its phase changes[16,18]. This provides an additional energy input to the atmosphere. The resulting positive feedback leads to an acceleration of the delayed ocean warming in low latitudes[19,20], to a strengthening of most tropical circulations and perhaps also to a greater frequency and/or intensity of warm ENSO events.

Because we have only short observation periods, we refrain from extrapolating these trends into the future. However, the search for a warming signal should be directed primarily at the tropics, instead of higher latitudes.

In the upwelling regions of the tropical oceans, the sea–air fluxes of CO_2 and H_2O are nearly simultaneously intensifying and weakening[24], during ENSO cycles. Among other considerations, this also suggests that the manmade increase in the atmospheric CO_2 concentration could be the primary trigger of a chain of processes within the tropical branch of the climate system. At present, we do not wish to speculate about the possible consequences of such an increase of the hydrological cycle in a key area of the climate system. We wish to bring the observed signal to the attention of others concerned with global warming. □

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Impact production of CO_2 by the Cretaceous/Tertiary extinction bolide and the resultant heating of the Earth

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EVIDENCE at the Cretaceous/Tertiary boundary suggests that the proposed 'extinction' bolide[1] struck a continental or shallow marine terrane. This evidence includes: shocked quartz and feldspar grains found in the boundary layer inherited from a range of rock types[2,3]; a high $^{87}Sr/^{86}Sr$ ratio in some planktonic fossils[4] which could reflect continental-derived Sr (ref. 5); and evidence that the platinum-group-element-rich clay layer is underlain (at some localities) by a deposit of possible tsunamic origin[6,7]. These observations and data demonstrate that sea level at the end of the Cretaceous was ~150–200 m higher than at present, suggesting the possibility that the extinction bolide struck a shallow marine carbonate-rich sedimentary section. Here we show that the impact of such a bolide (~5 km in radius) onto a carbonate-rich terrane would increase the CO_2 content of the atmosphere by a factor of two to ten. Additional dissolution of CO_2 from the ocean's photic zone could release much larger quantities of CO_2. The impact-induced release of CO_2, by itself, would enhance atmospheric greenhouse heating and give rise to a worldwide increase in temperature from 2 K to 10 K for periods of 10^4 to 10^5 years.

Many impact-induced phenomena have been suggested to exert a climatic effect. Alvarez *et al.*[1] proposed that the shielding of the Sun by dust injected into the atmosphere following a land impact would exert such an influence on global climate; this was later explored by others[8–10]. There is now good evidence that large amounts of soot, possibly from shock-induced forest fires, occur in the Cretaceous/Tertiary (K/T) layer[11]. An impact into the ocean can also induce a short-lived (< 0.1 year) greenhouse effect caused by water vapour[12]. The impact-driven atmospheric shock wave would produce large quantities of NO_x (refs 13–15), which could deplete the ozone layer through photochemical processes. Acidification of the atmosphere and land and ocean surfaces is also expected[16], which would result in increased atmospheric CO_2 upon dissolution from the ocean surface. Enhanced absorption of solar radiation by NO_x and CO_2 can affect the atmospheric thermal-radiation balance, and hence global climate.

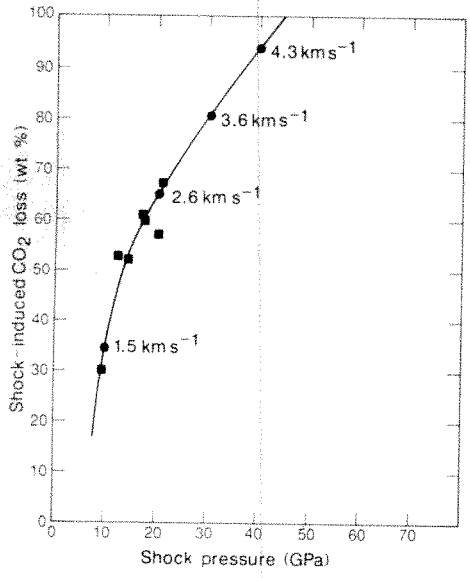


FIG. 1 Fraction of CO_2 released from calcite as a function of impact pressure[17] (squares). The solid line shows theoretical calculations, and corresponding impact velocities are indicated for shock pressures of 10, 20, 30 and 40 GPa (circles).

The amount of CO_2 released by impact-induced shock pressures in carbonate minerals can be estimated using the experimental results of Lange and Ahrens[17] and the impact scaling relations of O'Keefe and Ahrens[18]. Lange and Ahrens performed a series of laboratory impact experiments in which they measured the amount of CO_2 released as a function of peak shock pressure (Fig. 1). They found that the threshold shock pressure required to induce devolatilization of $CaCO_3$ to CaO and CO_2 is ~10 GPa.

The volume of vapour (V_v) relative to the volume of the impactor (V_m) is given by

$$\frac{V_v}{V_m} = 0.132\left(\frac{\rho_m}{\rho_s}\right)\frac{U^2}{\Delta E} \quad (1)$$

where ρ_m and ρ_s are the densities of the impactor and planetary surface, U is the velocity of impact and ΔE is the energy of vaporization; for silicates, $\Delta E \approx 0.2\times10^{12}$ erg g^{-1} (ref. 18). The effective energy of devolatilization of calcium carbonate was derived by scaling this energy of vaporization by the ratio of the pressures for incipient impact devolatilization:

$$\Delta E_{CaCO_3} = 0.2\times10^{12}P^v_{CaCO_3}/P^v_{sil} = 9.5\times10^9 \text{ erg g}^{-1} \quad (2)$$

where $P^v_{CaCO_3}$ and P^v_{sil} are the threshold pressures for incipient vaporization of calcite and silicates, respectively. We used this value of ΔE in equation (1) to calculate the volume of CO_2 produced.

The scaling relation given by equation (1) was derived from calculations of impact upon a homogeneous planet. Carbonate-rich sections are expected to range in thickness up to 4 km. For large-scale impacts (impactor radii > 5 km) the peak shock pressures are expected to completely devolatilize the carbonate-rich layer. Because the shock impedances of carbonate rocks are similar to those of silicates, the shock trajectories and pressures do not differ significantly between a homogeneous silicate planet and a carbonate sequence overlying a silicate half-space. With this assumption, the volume, V_L, of the carbonate layer that would be devolatilized is

$$V_L = \pi[R_v^2 d - \tfrac{1}{3}d^3] \quad (3)$$

where R_v is the radius of the region that is shocked to pressures sufficient for devolatilization and d is the mean thickness of the carbonate layer. Here R_v is given by

$$R_v = \left(\frac{6}{4\pi}V_v\right)^{1/3}$$

Our model has two layers: a carbonate-rich layer, which was assumed to be either 1 or 4 km thick, and an underlying silicate rock core. Less than one-half of the Earth's surface, comprising moderate-depth oceanic terrane or continental-shield terrane, is represented by a thin (1-km) carbonate layer, whereas the small fraction of the Earth's surface which has thick sections of shallow marine sediments[19] is represented by a 4-km layer. The total carbonate reservoir of the Earth, 7×10^{22} g, would provide a 400-m-thick layer covering the whole Earth[20]. We examined the effect of including an ocean with a depth of less than 3 km and concluded that for impactors with radii greater than 1 km, the ocean does not significantly affect the amount of CO_2 released. Our results for the mass of CO_2 evolved as a function of impactor size are shown in Fig. 2. Two types of impactors were considered; asteroids with a density of 3 g cm^{-3} and a velocity of 20 km s^{-1}, and comets with a density of 1 g cm^{-3} and a velocity of 60 km s^{-1}. The mass of CO_2 released exceeds

the present-day atmospheric content of 2.7×10^{18} g when the radius of asteroid impactor is greater than 3.1 and 6 km for a 4- and 1-km-thick carbonate layer respectively, and when the corresponding radius of a comet impactor is greater than 2.1 and 4.2 km. On the basis of the iridium content at the K/T boundary layer, Alvarez *et al.*[1] estimated the minimum radius of the K/T asteroid to be 5 ± 2 km. The radius of a cometary impactor must be 1.4 times that of an asteroidal impactor to give the same effect. For the above range of K/T impactor radii, it is clear that significant amounts of CO_2 can be ejected into the atmosphere.

In the case of impactors whose radii are of the order of a fraction of the atmospheric scale height (~7 km), the devolatilized CO_2 would be dispersed rapidly and globally. In contrast, the CO_2-depleted rock is expected to remain buried in the crater and its recarbonation must be retarded by the overlying ejecta blanket. Roddy *et al.*[21] and O'Keefe and Ahrens[22] have calculated the flow fields in the Earth and atmosphere that would result from the impact of a 5-km-radius bolide causing the K/T event. Although neither calculation included explicitly the effects of CO_2 devolatilization, it can be inferred from the geometry and evolution of the flow fields of the Earth and atmosphere that the evolved CO_2 would be propelled upward from the impact site and be distributed globally.

The sudden injection of CO_2 or water into the atmosphere will increase the Earth's mean surface temperature through the greenhouse effect[23]. The time required for the atmosphere to heat up by either mechanism is much less than the radiative cooling time (~3 days). The re-equilibration time of the solid-Earth/ocean/atmosphere system to a sudden large increase in CO_2 in the atmosphere is relatively long, whereas the re-equilibration time for an increase in atmospheric water is in the range of months to several years[12].

The re-equilibration of CO_2 by dissolution in the deep ocean water or dissolution of deep-sea sediments may occur on timescales of 10^3 to 10^4 years (ref. 24). Reaction with crustal rock is thought to occur through the rock-weathering reaction

$$CaSiO_3 + 2CO_2 + H_2O \rightarrow Ca^{2+} + SiO_2 + 2HCO_3^-$$

with Ca^{2+} and HCO_3^- subsequently removed by carbonate precipitation from sea water on a timescale of 10^5 years (ref. 25). Surface sea water is at present supersaturated with respect to bicarbonate ion, and an increase of the ocean surface temperatures, as well as production of NO_x and its subsequent uptake by the oceans, is expected to release additional CO_2 to the atmosphere[16,26].

We used the results of Kasting and Ackerman[27] to obtain the temperature rise at the Earth's surface as a function of CO_2 content and to calculate the temperature rise as a function of the size of the impactor from the conditions shown in Fig. 3. The increase in temperature as a function of mass of CO_2 in the atmosphere is given by $\Delta T = 10 \log_{10} m/m_0$, where m is the mass of CO_2 in the perturbed atmosphere and m_0 is the mass of CO_2 in the normal atmosphere[27]. The amount of CO_2 obtained in Fig. 2 is conservative, as it comes only from impacted carbonates and includes neither the effect of additional CO_2 expected from surface heating or acidification of the ocean[28], nor that induced by biomass destruction. Figure 3 indicates that the temperature change calculated is significant (>~5 K) for impactors greater than 3 km in radius. Emiliani *et al.*[12] have argued that the intolerance of large families of biota to increases in temperature of ~10 K would be sufficient to account for the K/T extinctions. Moreover, if such a temperature excursion remained for $>10^3$ yr, then global oceanic warming would also occur and major and complex changes in oceanic currents would result. This may be reflected in the generally unsteady and poorly correlated values of $\delta^{18}O$ measured for planktonic foraminifera tests over the first 10^5 yr of the Tertiary[29,30]. The most compelling evidence for a long period of extended CO_2-enrichment of the atmosphere, and by inference, an extended global temperature increase, stems from the 10^3–10^4-yr period of ^{13}C-depleted surface foraminifera tests and the concurrent absence of the usual relationship between heavy carbon ($\delta^{13}C = +3$ at the surface) and light carbon ($\delta^{13}C = +1$ at depth) measured in the normal biologically active ocean.

Emiliani *et al.*[12] and McLean[31] have also concluded from a palaeontological evidence of extinctions in marine fauna at the K/T boundary that the mean temperature increased at, or immediately after, the extinction event. We note that the decrease in $^{13}C/^{12}C$ ratio of the planktonic foraminifera appears to extend for 10^4–10^5 yr after the K/T event[32], in general agreement with the extinction timescale proposed by Hut *et al.*[33]. This is considerably longer than the time interval of $<10^3$ yr associated with deposition of the platinum-group-element layer. The extended 10^4–10^5-yr extinction interval is not easily explained by theories of aerosol atmospheric loading or by a water-induced greenhouse effect. Our calculations indicate that an enhanced atmospheric greenhouse effect caused by a large impact-devolatilization-induced increase in atmospheric CO_2 would result in significant heating of the Earth, causing a lengthy period of mass extinctions. □

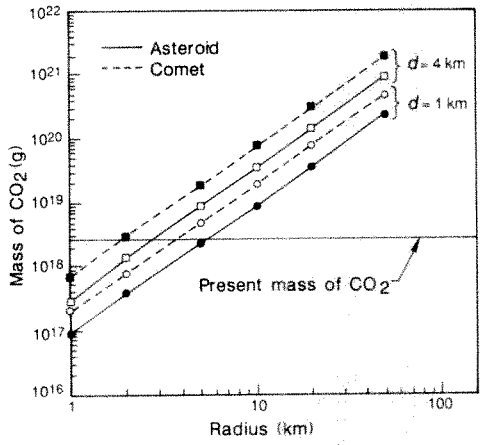


FIG. 2 Mass of CO_2 released during an impact from a carbonate sediment. The impact velocity is 20 km s^{-1} and the density of the impactor is 3 g cm^{-3} for an asteroid and 1 g cm^{-3} for a comet. The carbonate thickness, d, is either 1 or 4 km.

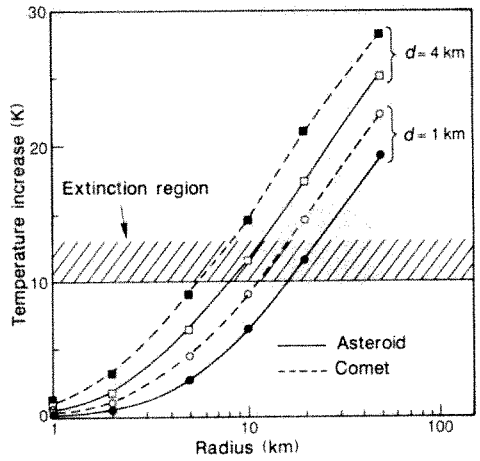


FIG. 3 Mean rise in atmospheric temperature as a function of impactor radius. Impact conditions are described in Fig. 2. The extinction region[1,2] is indicated.

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Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting

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INDIVIDUALS are often assumed to behave so as to maximize their reproductive success[1] but unambiguous determination of parentage is difficult, especially in species with complex social systems where a female may mate with several males and where there may also be intraspecific brood parasitism[2–4]. Even in apparently monogamous species, extra-pair paternity can be common[5–7]. DNA fingerprinting[8–11] promises to revolutionize field studies by providing a powerful method for determining paternity and maternity[12]. Here we use this technique to link observations of mating behaviour and parental care with precise measurements of reproductive success. We show that in the dunnock *Prunella modularis*, a small passerine bird with a variable mating system[13,14], males do not discriminate between their own young and those of another male in multiply-sired broods. Nevertheless, they increase their own reproductive success by feeding offspring in relation to their access to the female during the mating period, which is a good predictor of paternity.

We studied a colour-ringed breeding population of about 80 dunnocks in the Cambridge University Botanic Garden[14–16]. Some female territories were defended by one male (monogamy), others by two, unrelated males (polyandry). Sometimes two males jointly defended the territories of two or three adjacent females (polygynandry). Where two males shared the defence of one or more female territories, one (alpha) was clearly dominant and able to displace the other (beta) from the female's vicinity[14].

In 1988 we observed females from the time they completed nest building until the start of incubation (6–10 days), which is the period when copulations occur[15,16] (mean observation time 4.02 h, $N = 34$). Monogamous males spent on average 81.1% of their time within 10 m of the female (range 62.1–97.3%, $N = 10$). Mate guarding was very effective; intruders were seen to gain exclusive access to the female (defined as the presence of only one male within 10 m) in only 3 of the 10 cases, a total of only 3 min in 23.98 h observation (mean for the 10 cases = 0.17% observation time).

By contrast, there was intense competition for matings when two males shared a female. Alpha males attempted to guard the female and prevent the beta male from gaining access, but this was difficult because beta males were often very persistent and, furthermore, females attempted to escape the alpha males' close attentions and encouraged beta males to mate[16]. In 8 out of 11 cases of polyandry and 10 out of 13 cases of polygynandry, the beta male had some exclusive access to the female. There was no significant variation in the proportion of exclusive access gained by alpha or beta males at different stages of the mating period and the mean copulation rate during periods of exclusive access was no different for alpha males (1.98 per h) and beta males (2.15 per h; $N = 16$; Wilcoxon matched pairs test, NS). Exclusive access time therefore gave a good measure of mating success and was easier to record than copulations. As in monogamy, intruding males gained very little exclusive access (only 3 out of 24 cases, a total of 5 min in 112.7 h; mean of 0.05% observation time).

As expected from our mating observations, DNA fingerprinting (Fig. 1) shows that intruding males gained little success; of 133 young (45 broods) all but one were fathered by a resident male. The exception is a case of polyandry where one chick from a brood of four was fathered by an alpha male from a neighbouring territory. All chicks from monogamous mating systems were sired by the resident male, whereas in polyandry and polygynandry paternity was often shared between alpha

TABLE 1 Analysis of paternity by DNA fingerprinting for three dunnock mating systems

Mating system	% Total young (*n*) fathered by		Mean % paternity per nest (*n*)		Details for each nest: proportion fathered by $\alpha\delta$
	$\alpha\delta$	$\beta\delta$	$\alpha\delta$	$\beta\delta$	
Monogamy (1♂ 1♀)	100	— (49)	100	0 (15)	4/4, 4/4, 4/4, 4/4, 4/4, 4/4, 3/3, 3/3, 3/3, 3/3, 3/3, 3/3, 3/3, 2/2, 2/2.
Polyandry (2♂ 1♀)	52.9	44.1 (34)*	49.2	48.5 (11)*	4/4, 3/4*, 3/3, 2/2, 2/4, 1/4, 1/4, 1/3, 1/3, 0/2, 0/1.
Polygynandry (2♂ 2♀; 2♂ 3♀)	72.0	28.0 (50)	68.4	31.6 (19)	3/3, 3/3, 3/3, 3/3, 3/3, 2/2, 2/2, 2/2, 1/1, 3/4, 3/4, 2/4, 2/4, 2/3, 1/3, 1/2, 0/2, 0/1, 0/1.

The proportions of young sired by the two males in polyandry and polygynandry are not significantly different. All but one of the 133 young were fathered by a male resident on the territory.

* One chick was fathered by a male on a neighbouring territory (see Fig. 1 legend).

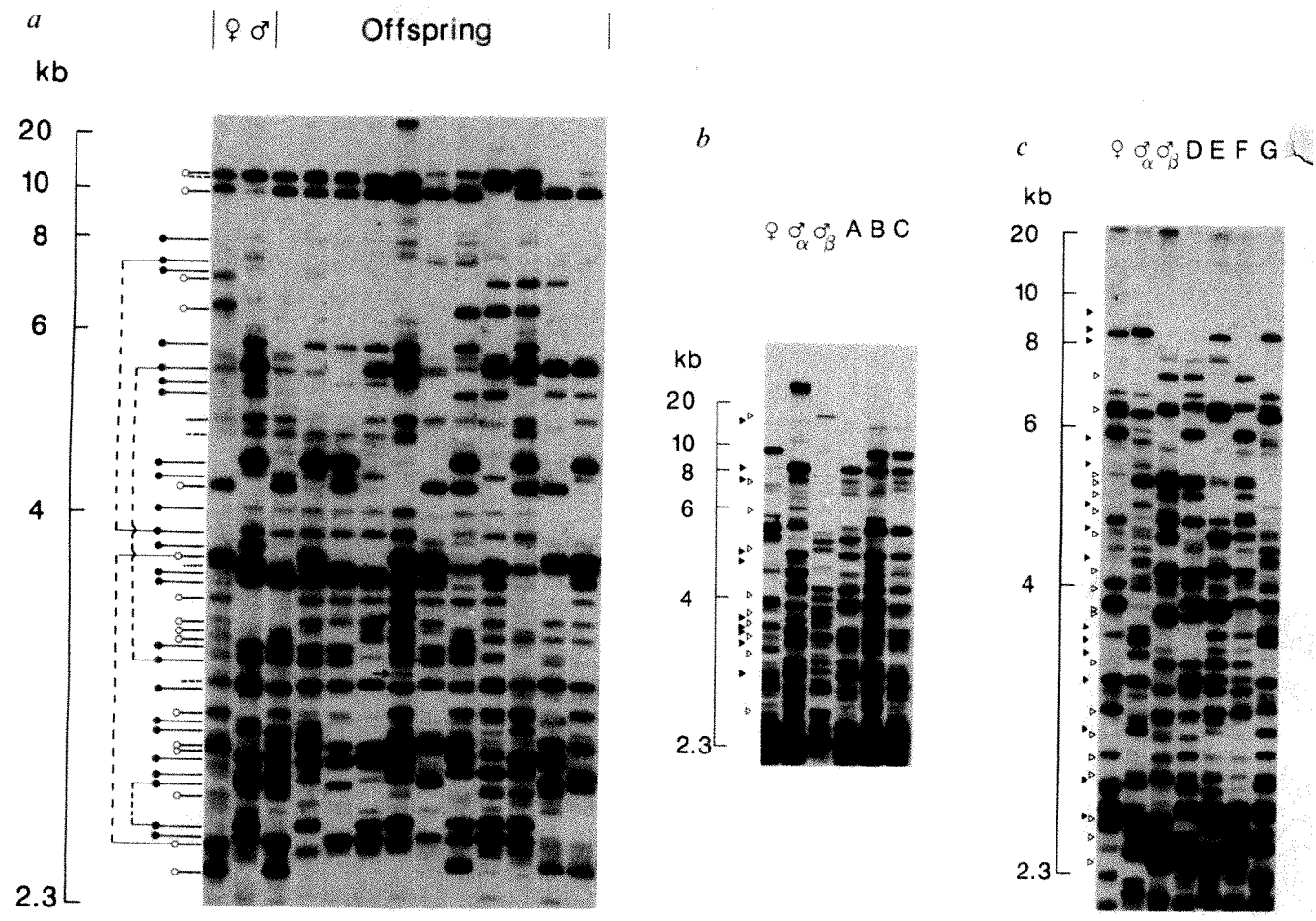


FIG. 1 Example dunnock DNA fingerprints obtained with probe 33.15 (ref. 8). *a*, Monogamous pair and 11 offspring (3 broods). DNA fragments of 'almost identical' (·····) and 'apparently identical' (– – –) mobility are indicated. Segregation analysis[9,17] of the other 40 resolved maternal (○) and paternal (●) heterozygous fragments larger than 2.3 kb revealed a transmission frequency of 0.495, four apparently allelic pairs (joined by – – –), and no consistent cosegregation; hence the bands probably represent dispersed hypervariable minisatellite DNA sequences[9,17]. *b*, Polyandry (exclusive paternity): brood where alpha male sired all three nestlings A, B, C. *c*, Polyandry (mixed paternity): brood where beta male sired nestlings D, E, F, and alpha sired G. Markers indicate paternal male-specific (diagnostic) alpha (►) and beta (▷) bands.

METHODS. DNA was extracted from blood (40–100 μl, from brachial veins of adults and 7-day-old nestlings into 1×SSC, 10 mM EDTA pH 7.0), digested with *Alu*I, and fingerprints obtained as previously described[9] except: 5 μg ethidium bromide was added per sample, gels were 0.8% (20-cm long) or 1.0% (30-cm long) agarose in 1×TBE, and hybridizations, at 64 °C, excluded competitor DNA. The probability of band sharing (x) between individuals[9] (conservatively found from 20-cm gels, including 'almost identical' bands) = 0.24 ± 0.10 s.d. (N = 17); bands per fingerprint (n) = 23.2 ± 4.1 s.d. (N = 29); hence mean allele frequency[18] (q) = 0.13. For trios, fathers were initially assigned by the presence of diagnostic bands in each nestling (range 2–13, mean 7.4 ± 2.5 s.d., N = 75). In three nestlings, one or two (arrowed in *a*) bands absent in both parents were considered mutant[19] as band sharing was otherwise greater than expected for a misassigned random adult ($P_1 < 0.01$, $P_2 < 0.001$, $P_3 < 0.05$); apparent mutation rate = 10^{-4}. For the other 130 offspring $n \geq 12$; it is extremely unlikely that all 12 fragments would be present in a random pair[20] as $P = (1-(1-x)^2)^n = 3.3 \times 10^{-5}$. We concluded that no intraspecific brood parasitism, at least by non-relatives, occurred and that all mothers were correctly identified. Obligate paternal-derived bands could therefore be inferred in all offspring (range 5–18, mean 9.9 ± 2.8 s.d., N = 133). The probability of an unrelated male containing all these m fragments, $P = x^m$, in each case lay between 8.0×10^{-4} (m = 5) and 7.0×10^{-12} (m = 18); similarly for a close relative (brother, father and son)[21], $P = ((1+x)/2)^m = 0.092$ to 1.8×10^{-4}. Demographic data[14] also shows that close relatives are very unlikely to become neighbours. In the one case of a nestling sired by an intruding male the female shared 14 of 25 offspring bands ($P < 0.001$) and the assigned male contained all 11 paternal specific bands ($P < 2 \times 10^{-7}$), which is also significantly more than if he were the actual father's brother (expected band sharing[9] = $1-(1-((1+x)/2(2-q)))(1-q) = 0.42$, $P < 0.001$).

TABLE 2 Association between paternity, feeding of nestlings and access to the female during the mating period for alpha and beta males in polyandry and polygynandry

Paternity (from DNA fingerprinting)	Behavioural observations: Male fed young	Male did not feed young	Male had some exclusive access to female	Male did not have exclusive access to female
Alpha male				
Broods where he had paternity	23	1	13	0
Broods where he had no paternity	2	3	4	1
	P = 0.011*		Sample size too small to test	
Beta male				
Broods where he had paternity	14	3	10	0
Broods where he had no paternity	4	8	2	6
	P = 0.011*		P = 0.005*	

Chick feeding by males was recorded for 29 out of the 30 nests in Table 1; we had observations during the mating period for 18 of these cases. We recorded 90% cases where beta males fed the young, and 85% cases where they had some exclusive mating access, within the first hour of observation, so observation times (see text) were sufficient to give reliable measures.

* Fisher exact probability test.

nd beta male (Table 1). Maternity was assigned in all cases to ıe resident female, so there were no cases of intraspecific brood arasitism.

In all 15 cases of monogamy, the young were fed only by the ıonogamous male and female. In the 30 cases of polyandry nd polygynandry where we recorded chick feeding, the female ed the young at all 30 nests, helped by both males in 14 cases, y the alpha male only in 11 cases (in one of these the beta ıale died during incubation), by the beta male only in 4 cases in one of these the alpha male died) and in one case the female ot no male help (mean observation time per nest = 4.35 h, V = 45).

DNA fingerprinting showed that males were more likely to ıelp feed the young if they had paternity (Table 2). Two results ndicate that this association does not arise because males can ecognize their own offspring. First, in two cases the alpha male ed nestlings even though he had no paternity and in four cases he beta male did so. (In two cases both helped to feed a single :hick.) Second, after fledging, broods were often divided among he parents with each adult taking sole care of some young for ı further 2 weeks until they became independent. There was no endency for males to pick out their own young. We observed 28 dependent fledglings, from 12 broods where both alpha and ɔeta males had helped to feed the nestlings; 11 young were sired ɔy the alpha male, of which 5 were fed by their father and 6 by :he beta male, whereas 17 were sired by the beta male, of which 9 were fed by their father and 8 by the alpha male.

How, then, does the association between chick feeding and paternity come about? A male's access to the female gives a good prediction of his chances of paternity (Table 2), and males apparently use mating access to determine whether they feed young. Including data from all polyandrous and polygynandrous mating systems observed in 1981–88, beta males fed the young in 22 of the 27 cases where they had some exclusive access during the mating period, compared with only 1 out of 11 cases where they were not observed to have access ($P < 0.001$). Furthermore, beta males brought a greater proportion of the male feeds where they had had a greater proportion of the exclusive access time during the mating period (Fig. 2).

Females benefit by copulating with two males because chicks are fed more, and more survive with the help of two males rather than one[22]. An alpha male, however, would need at least 60–70% of the paternity in trio-fed broods for co-operative polyandry to bring greater reproductive success than monogamy[23]. For 20 broods where both males in polyandry and polygynandry had access to the female during the mating period, or both fed the young (and so, by implication, both had had access), the mean paternity split was 55.0% alpha, 45.0% beta (total of 58 young, 56.9% sired by the alpha male). This suggests that alpha males do not achieve the critical proportion above which it would pay them to share the female, and explains why although females encourage the presence of beta males, alpha males attempt to drive them away[23]. □

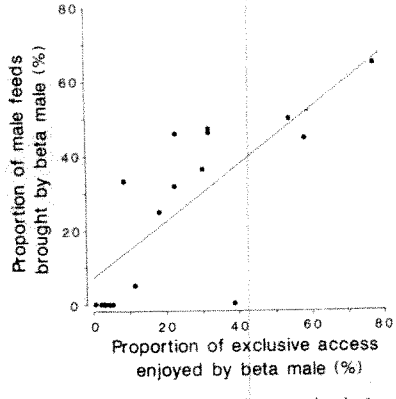


FIG. 2 Relationship, for polyandry and polygynandry, between the proportion of exclusive access time with the female by the beta male during the mating period, and the proportion of male feeds brought by the beta male to the nestlings. Exclusive access time is the total time that either the alpha or beta male was the only male within 10 m of the female. Data from 1981–88 for cases where beta males gained some exclusive access ($y = 5.75 + 0.798x$: analysis on log-transformed data: $r = 0.775$, 15df, $P < 0.01$; the slope does not differ significantly from 1, $t = 1.33$, 15df). In polygynandry, a male's provisioning of the young at one nest may be influenced by the activities of other females in the mating system,[16,22] so cases where other females were laying or had young simultaneously were excluded.

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Incubation period of AIDS in San Francisco

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IN a closed population, the distribution of AIDS diagnoses over time is the convolution of the distributions of human immunodeficiency virus (HIV) infections and the incubation period. This has motivated estimates of the infection distribution, assuming known diagnosis and incubation distributions[1], but the usefulness of this method is limited by uncertainty about incubation[2–4]. The large amount of information on the distribution of HIV infections in San Francisco's gay community suggests the opposite approach—estimating the incubation distribution, assuming known infection and diagnosis distributions. A non-parametric implementation of this strategy produced an estimate with a median at 9.8 years, increasing hazard rates, and less uncertainty than previous estimates.

Data are available for seroconversions rather than infections, so we estimated the time from seroconversion to AIDS diagnosis[5]. (Seroconversion may occur later than infection[6].) Figure 1*a* shows estimated seroconversion rates from three sources, and Fig. 1*b* shows the overall seroconversion and diagnosis patterns that we used. The dramatic drop in seroconversion by 1984 was paralleled by rectal gonorrhea rates among males at the same time[7], presumably because of behaviour change. The effect of such change on HIV infections would have been enhanced by the saturation of high-risk subgroups[8]. This further

TABLE 1 Estimates of the incubation period of AIDS

Estimate	Median (yr) (95% CI)	Per cent with AIDS by 9 yr (95% CI)
Present study	9.8 (9.4, ∞)	45 (42, 47)
Penalized 15% for uncertainty in seroconversion pattern	(8.6, ∞)	(36, 54)
Parametric analysis of US transfusion data[3]	7.3 (4.6, ∞)	Not given
Parametric analysis of data from 84 men in the San Francisco City Clinic Cohort[2]	7.8 (4.2, 15.0)*	65 (not given)
Nonparametric analysis of data from 181 men in the San Francisco City Clinic Cohort[15]	Not given	42 (30, 54)†

* Estimated mean and 90% confidence interval (CI).
† Confidence interval does not reflect uncertainty resulting from imputing exact seroconversion times from interval-censored data.

corroborates the drop shown by all three sources in Fig. 1*a*. Mathematical models of epidemic spread in a susceptible population[8] corroborate the early rapid rise.

Treating the distributions in Fig. 1*b* as known allows estimation of the incubation distribution. In particular, we wish to estimate parameters h_k, the hazard rates for developing AIDS k months after seroconversion. With 10 years worth of data, h_k can be estimated for $k=0$ to 120. Let $d_{j,k}$ be the number of persons who seroconverted in month j and were diagnosed in month k. If all the $d_{j,k}$ were known, then the maximum likelihood estimate of h_k would be

$$\hat{h}_k = e_k / r_k \qquad (1)$$

where $e_k = \sum_{j=0}^{120-k} d_{j,j+k}$ is the number of persons diagnosed exactly k months after seroconversion, and r_k is the number of persons still free of AIDS $(k-1)$ months after seroconversio Although the $d_{j,k}$ are unknown, we can use the EM algorithm to estimate the h_k. The algorithm begins with arbitrary initi values of the h_k and calculates the expected values of the d conditional on the initial h_k and the known seroconversion an diagnosis patterns. The expected values are used in place of t unknown $d_{j,k}$ in equation (1) to yield refined estimates of th h_k. The refined h_ks lead to new expected values, which yiel new estimated h_ks, and so on. The algorithm continues unt the estimates converge.

Because it is biologically reasonable that the hazard change smoothly over time, we used a modification of equation (1):

$$\hat{h}_k = \sum_{i=k-6}^{k+6} e_i \Big/ \sum_{i=k-6}^{k+6} r_i \qquad (2$$

This is a local likelihood estimate[10] with a span of 13. (Thi span was the smallest that produced acceptably smooth esti mates.) In addition to ensuring reasonable smoothness, thi modification also reduced the estimate's variability and led t faster convergence, while still being non-parametric. Figure shows the estimates that resulted from applying this method t the distributions in Fig. 1*b*. The estimated probabilities o developing AIDS during the first to tenth years followin seroconversion were 0.002, 0.007 0.022, 0.043, 0.061, 0.073, 0.082 0.081, 0.074 and 0.067, respectively. The estimated distributior is an average for all gay men in San Francisco. It may well diffe for subgroups of this population, and it may change as prophy laxis of seropositives becomes more widespread before diag nosis.

The confidence intervals in Fig. 2 do not reflect the uncertainty that results from estimation of the seroconversion pattern. Multiplying the seroconversion curve in Fig. 1*b* by a factor (or dividing the diagnosis curve by a factor) would divide the curve in Fig. 2*a* by the same factor. As the estimated number of seroconversions may be too high or too low by 10% (ref. 11), the curve in Fig. 2*a* could plausibly be multiplied by 0.9 or 1.1.

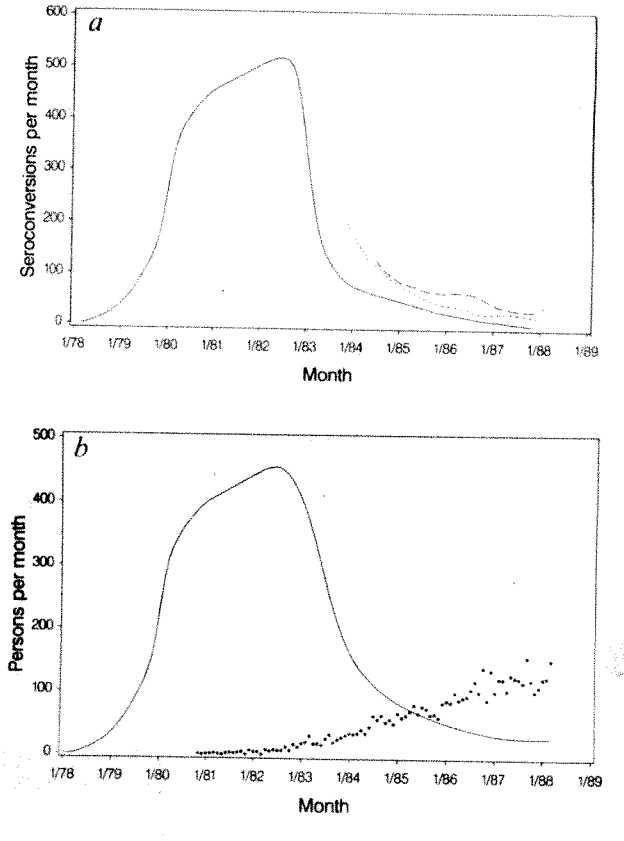
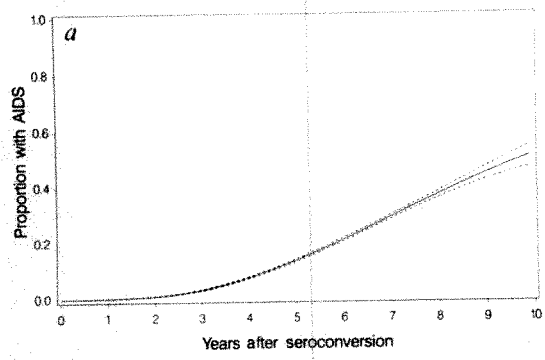


FIG. 1 Data used for estimating the distribution of times from HIV seroconversion to AIDS among gay men in San Francisco. *a*, HIV seroconversions estimated from the hepatitis B vaccine trial (solid line), the San Francisco Men's Health Study[11] (long dashes), and the San Francisco General Hospital Study[12] (short dashes). *b*, Overall estimates of HIV seroconversions (solid line) and AIDS diagnoses (dots).
METHODS. *a*, For the hepatitis B vaccine trial, a smooth curve was fitted to match estimated yearly totals[16] and scaled to total 20,260 seroconversions through September 1984. The total 20,260 is an estimated 8,757 seropositive but AIDS-free men from a random sample of part of San Francisco[11], divided by the proportion of AIDS cases from October 1984 until July 1988 who came from the sampled area (1,708 of 3,767, or 45%; this proportion was fairly stable over the period) plus 800 AIDS cases diagnosed up to September 1984. For the other cohorts, Turnbull's EM algorithm[13] was applied to interval-censored seroconversion data, with local-likelihood smoothing[10] in the *M*-step. The curves were scaled by the same factor as the vaccine trial estimate. *b*, Monthly diagnosis totals up to March 1988 are from surveillance data as of July 1988, adjusted for several sources of error. Based on data reported of November 1987 until July 1988, the proportionate increase in monthly totals arising from reports more than four months after diagnosis was modelled as a quadratic function of difference between report and diagnosis month, plus a quadratic function of calendar month of report (to allow for diminishing influence of the change in case definition[5]). This yielded corrections ranging from +14% for diagnoses in March 1988 to less than +1% for March 1987 and before. The San Francisco Department of Public Health has estimated under-reporting as ~2% before 1986 and ~5% more recently, and has also estimated that about 4% of San Francisco residents with AIDS are diagnosed elsewhere (unpublished data). Follow-up of the San Francisco General Hospital Cohort[12], in which 3 of 69 AIDS cases (4%) were diagnosed after moving from the city, suggested a correction of +4% for persons included in the seroconversion totals who were not residents when diagnosed. The seroconversion curve is a compromise among the curves from (*a*) that retains the overall shape from the hepatitis B vaccine trial and agrees closely with the other curves, while remaining smooth and totalling 20,260 seroconversions up to September 1984.



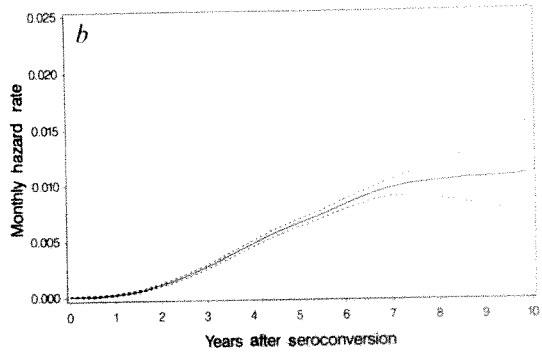


FIG. 2 Estimated distribution of time from HIV seroconversion to diagnosis of AIDS for gay men in San Francisco, 1978–1988. *a*, Cumulative distribution function (solid line), with approximate 95% confidence intervals (dashed lines). *b*, Hazard function (solid line), with approximate 95% confidence intervals (dashed lines).
METHODS. Confidence intervals were determined by a bootstrap method[14]. The estimated incubation distribution was used to generate simulated diagnosis data assuming the seroconversion pattern shown in Fig. 1*b*. Estimates were made on 200 such sets of simulated data to assess the variability resulting from random variation in how well the diagnosis pattern reflects the incubation distribution. At each month, 95% of the estimates fell between the dashed lines.

Shifting the seroconversion curve relative to the diagnosis curve would expand or contract the initial period of virtually no progression to AIDS. Applying the estimation method to a seroconversion pattern identical to the one in Fig. 1*b*, but with 10 seroconversions per month from December 1976 to May 1978, would change the estimate in Fig. 2*a* by less than 0.006 at all time points, so the possibility of an unobserved long, slow start to the epidemic does not invalidate the estimate.

Figure 3 compares fitted monthly AIDS diagnosis totals with adjusted surveillance data. The fitted curve matches the data well. The projected curves reflect the range of distributions shown in Fig. 2*b*. They are not influenced by uncertainty in the magnitude of the seroconversion curve, because any error would result in an opposite error in the incubation estimate, with no net influence on projected totals. The projections show the sharp drop in seroconversions in Fig. 1*b* manifesting itself as a much more gradual drop in AIDS diagnoses 6 to 8 years after the drop in seroconversions. For each of the three projections, the time between the peaks in seroconversions and projected diagnoses is approximately equal to the time of the peak in the density of the incubation. The gradualness of the drop in projected diagnoses is due to the large variability in incubation times[8].

Four factors may bias the estimate of Fig. 2 upwards when compared with other studies: use of the more inclusive 1987 definition of AIDS[5], use of seroconversion rather than infection times, inclusion of cases who seroconverted outside San Francisco in diagnosis totals but not in seroconversion totals, and possible downward bias in the magnitude of the seroconversion curve resulting from over representation of seropositives from the sampled area[11] among diagnosed AIDS cases. Despite these factors, Fig. 2 shows slower progression to AIDS than previous estimates[2,3] (Table 1). The large uncertainty in previous estimates, however, makes them quite compatible with Fig. 2. In addition, analyses based on blood-transfusion data deal with a very different population and only estimate the incubation period that is conditional on AIDS developing.

The incubation period of AIDS is difficult to study. Prospectively followed incident seroconverters are rare, so estimates based on them are highly uncertain[2], and data from transfusion-associated AIDS cases are inherently limited[3]. The method used here is an alternative approach. It is possible because of the availability of information on seroconversions among gay men in San Francisco and because the drop in seroconversions makes the pattern of diagnoses quite informative about the incubation. Its dependence on an accurate estimate of seroconversions is a drawback, but it does not rely on parametric assumptions, and Table 1 shows that the estimate is more precise than others, even allowing for uncertainty in estimating seroconversions.

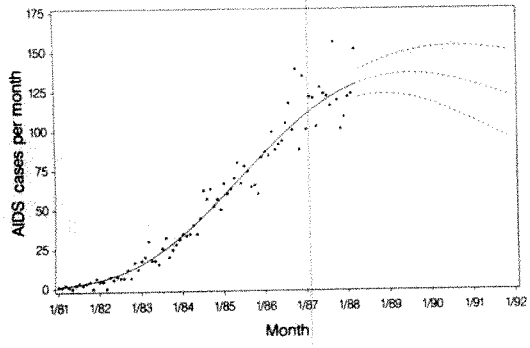


FIG. 3 Fitted (solid line) and projected (dashed lines) AIDS diagnoses among gay men in San Francisco. Projections are based on incubation distributions corresponding to the estimate in Fig. 2*b*, with the hazard remaining constant beyond year 10 (middle projection), and corresponding to the upper and lower confidence limits in Fig. 2*b*, with the hazard extrapolated linearly beyond year 10 (upper and lower projections). The adjusted surveillance totals from Fig. 1*b* (dots) are shown for comparison. Note that the vertical scale represents corrected rather than raw surveillance totals.
METHODS. Given v_j seroconversions in month j and probability p_k of developing AIDS k months after seroconversion, the expected number of diagnoses in month i is $\sum_{j=0}^{i} v_j p_{i-j}$. Fitted and projected monthly diagnosis totals were calculated by this formula with the v_js from the seroconversion curve in Fig. 1*b* (with constant extrapolation through 1991) and the p_ks from the incubation distributions described above.

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The HIV-1 *rev trans*-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA

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HUMAN immunodeficiency virus type 1 (HIV-1) replication requires the expression of two classes of viral mRNA. The early class of HIV-1 transcripts is fully spliced and encodes viral regulatory gene products. The functional expression of one of these nuclear regulatory proteins, termed Rev (formerly Art or Trs), induces the cytoplasmic expression of the incompletely spliced, late class of HIV-1 mRNAs that encode the viral structural proteins, including Gag and Env[1–6]. Here, we provide evidence that this induction reflects the export from the cell nucleus to the cytoplasm of a pool of unspliced viral RNA constitutively expressed in the nucleus. The hypothesis that Rev acts on RNA transport, rather than splicing, is further supported by the observation that the cytoplasmic expression of a non-spliceable HIV-1 *env* gene sequence is also subject to Rev regulation. Here we show that this Rev response requires a specific target sequence which coincides with a complex RNA secondary structure present in the *env* gene. The response to Rev is fully maintained when this sequence is relocated to other exonic or intronic locations within *env* but is ablated by inversion. These results indicate that the HIV-1 *rev* gene product induces HIV-1 structural gene expression by activating the sequence-specific nuclear export of incompletely spliced HIV-1 RNA species.

In the absence of Rev, the genomic HIV-1 *tat* gene expression vector pgTAT[6] (Fig. 1*a*) predominantly expresses a fully spliced cytoplasmic *tat* mRNA (Fig. 1*b*, lane 9) that encodes the 86-residue form of the HIV-1 Tat protein (Fig. 1*c*, lane 1). In the presence of Rev, pgTAT produces a high level of an unspliced cytoplasmic mRNA (Fig. 1*b*, lane 10), structurally equivalent to *env* mRNA[6], that encodes a truncated, 72-residue Tat protein (Fig. 1*c*, lane 2). Because it has been proposed that Rev suppresses the splicing of HIV-1 transcripts[1,7], we examined the effect of substituting heterologous splice donor (pInsSD) or splice acceptor and branch-point sequences (pInsSA) for the authentic pgTAT splice sites (Fig. 1*a*). In each case, these sequences were derived from the genomic rat preproinsulin II gene[8], which is not itself responsive to Rev (see below). Nuclease S_1 protection analysis using probes which spanned the insulin gene splice donor (Fig. 1*b*, lanes 1–4) or splice acceptor (Fig. 1*b*, lanes 5–8) reveals that the transfected pInsSA and pInsSD vectors predominantly express spliced cytoplasmic mRNA in the absence of Rev. Although these chimaeric transcripts used the predicted insulin RNA splice sites (Fig. 1*b*, lanes 1, 3, 6, 7) co-expression of *rev* nevertheless induces a high level of unspliced cytoplasmic mRNA (Fig. 1*b*, lanes 4, 8). In the case of pInsSA, the induced expression of an unspliced cytoplasmic *tat* mRNA was confirmed by immunoprecipitation analysis (Fig. 1*c*, lanes 3, 4).

The retention of full Rev responsiveness by pInsSA and pInsSD showed that the *cis*-acting Rev response element (RRE) must lie within the intronic *env* sequences of pgTAT. A series of deletion (pΔ) mutants of pgTAT (Fig. 1*a*) revealed that vectors retaining an intronic *Bgl*III (B2) to *Hin*dIII (Hd) fra ment also retained full Rev responsiveness (Table 1). All vectc lacking this sequence, however, exclusively express the 8 residue Tat encoded by the spliced *tat* mRNA, regardless of r co-expression. To localize more precisely the RRE within t B2/Hd fragment, we reinserted a series of subfragments in pΔB2/Hd to generate the pi vectors (Fig. 1*a*). The responsiv ness of these vectors to Rev (Table 1) showed that the RR must be at least 104 and possibly more than 210 nucleotid (nt) in length (Fig. 1*a*). Reinsertion of the complete B2/H fragment in the inverted orientation (piH/A) fails to restore R responsiveness (Fig. 1*b*, lane 16).

To determine whether the function of the RRE depends c location, we reinserted the B2/Hd fragment at selected sit within the Rev non-responsive pΔB2/Hd vector (Fig. 1*a*). The relocation (pλ) vectors reveal that the RRE is fully function both at other intronic sites (pλK and pλB1, Table 1) and whe inserted at an exonic *Bam*HI (Bm) site located 3′ to the *tat* gen splice acceptor (pλBm) (Fig. 1*c*, lanes 9, 10). Insertion at a *Eco*RI (R) site located immediately 3′ to the vector polyadenyl tion site (pλR) however, did not restore Rev responsiveness t pΔB2/Hd (Fig. 1*c*, lanes 11, 12). It seems therefore that th RRE can function at any location within the primary *tat* gen transcript when present in the sense orientation.

The minimal size of the RRE is relatively large, indicatin that secondary structure, in addition to primary sequence, ma be important for recognition of the RRE. Indeed, the sequenc encoding the N terminus of the HIV-1 gp41 Env protein i consistent with a high level of secondary structure[9], and in Fig 2 we present a stable predicted RNA stem-loop structure, whicl fully encompasses the minimal RRE defined by mutationa analysis. The extension of this 234-nt secondary structure beyon the minimal 210-nt RRE defined by piD/G and piB/G ma explain the incomplete Rev responsiveness of these vector (Table 1). Interestingly, the only other predicted structure o comparable significance within the HIV-1 genome coincide with the putative RNA target sequence for *trans*-activation b the viral *tat* gene product[9–11].

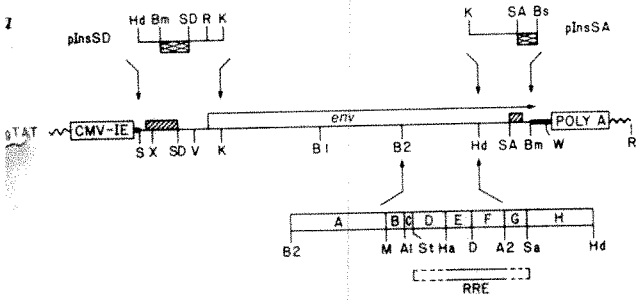
Although the data indicate that the *rev* gene product regulate the cytoplasmic expression of unspliced HIV-1 mRNA b directly or indirectly interacting with an RNA secondary struc ture, the observation that the RRE can function at both exonic and intronic locations argues against a direct effect on viral

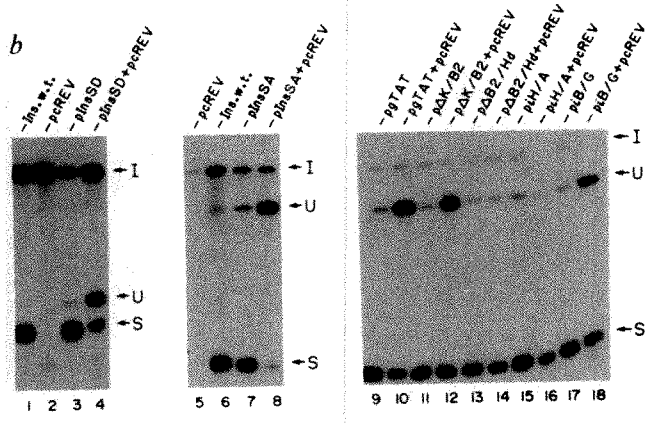
TABLE 1 Mutational definition of the HIV-1 Rev response element

Clone tested	Rev response	Clone tested	Rev response
pgTAT	+++	Reinsertions	
Substitutions		piA/H	+++
pInsSD	+++	piB/H	+++
pInsSA	+++	piB/G	++
Deletions		piB/E	—
pΔK/B1	+++	piC/F	—
pΔK/B2	+++	piD/G	++
pΔK/Hd	—	piA/D	—
pΔB1/B2	+++	piE/H	—
pΔB1/Hd	—	Inversions:	
pΔB2/Hd	—	piH/A	—
Relocations:			
pλK	+++		
pλB1	+++		
pλBm	+++		
pλR	—		

Different mutants of the parental pgTAT vector (Fig. 1*a*) were assayed for the Rev-induced expression of unspliced cytoplasmic *tat* mRNA by S_1 used elsewhere analysis (Fig. 1*b*) and immunoprecipitation (Fig. 1*c*). +++, full Rev response; ++, partial Rev response; —, no detectable Rev response. All constructions displayed the same phenotype as pgTAT in the absence of Rev coexpression.

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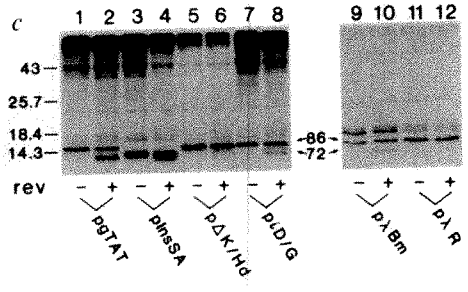


FIG. 1 Mapping of the HIV-1 Rev response element (RRE). *a*, The Rev responsive *tat* expression vector pgTAT contains the two HIV-1 *tat* gene-coding exons (hatched) separated by an *env*-gene-derived intronic element[6]. pInsSD and pInsSA were constructed by replacement of the authentic HIV-1 splice donor and splice acceptor elements with equivalent sequences from the genomic rat preproinsulin II gene expression vector pBC12MI[8]. Deletion (pΔ) mutants were constructed by excision of sequences between intronic restriction-enzyme sites. The pΔB2/Hd deletion mutant served as substrate for construction of the reinsertion (pi) and relocation (pλ) mutants. The names of the pi derivatives indicate which inclusive subfragment was reinserted. The pλ mutants were constructed by insertion of the complete A/H fragment at novel sites indicated by the vector name. The pENV160 vector lacks the HIV-1 SD sequence and instead proceeds from an *Ava*II (V) site located immediately 5′ to the *env* gene ATG[3]. pENV160 also contains 3′ *env* gene sequences which overlap with, and extend 3′ to, *rev* gene-coding sequences deleted from pgTAT. HIV-1 sequence coordinates (strain HXB-3) are relative to the start of the HIV-1 sequence at *Sal*I at position 5,367 (ref. 18). S, *Sal*I, 2; X, *Xho*II, 58; SD, splice donor, 260; V, *Ava*II, 387; *env* initiation codon, 482; K, *Kpn*I, 564; B1, *Bgl*II, 1,254; B2, *Bgl*II, 1,834; M, *Mbo*II, 1,957; Al, *Alu*I, 1,986; St, *Sty*I, 1,994; Ha, *Hae*III, 2,058; D, *Dde*I, 2,103; A2, *Alu*I, 2,162; Sa, *Sau*3AI, 2,201; Hd, *Hin*dIII, 2,354; SA, Splice acceptor, 2,590, Bm, *Bam*HI, 2,688, Bs, *Bst*EII; W, *Alw*I; R, *Eco*RI. *b*, Quantitative S_1 nuclease protection analysis of cytoplasmic RNA isolated from COS cell cultures transfected with the indicated expression vectors[6,8]. Transfections were performed in the presence of the *rev* expression vector pcREV or the negative control vector pBC12/CMV[6,8]. The end-labelled, input probes (I) were designed to permit visualization of both unspliced (U) and spliced (S) cytoplasmic mRNA species. Ins. w.t., cultures transfected with the wild-type preproinsulin II gene expression vector pBC12MI (ref. 8). *c*, Immunoprecipitation analyses of transfected COS cell cultures were performed using anti-*tat* antisera[6]. Vectors were transfected in the presence (+) or absence (−) of the *rev* expression pcREV (ref. 6). The spliced pgTAT mRNA encodes an 86-residue protein whereas the unspliced mRNA encodes a 72-residue protein[6]. pInsSA lacks the second *tat* exon (Fig. 1*a*) and encodes a spliced mRNA predicted to give an 84-residue chimaeric Tat protein.

METHODS. *b*, The strategy for the S_1 nuclease protection analyses has been described[6]. In each case, the DNA probe was end-labelled within a coding exon and extended into the flanking intron. A pBR322-derived tag permits the distinction of the input probe from the unspliced RNA signal. The probe used in Fig. 1*b*, lanes 1–4 was labelled at an insulin gene *Bam*HI (Bm) site using Klenow DNA polymerase I (Fig. 1*a*) and extends through the insulin gene splice donor (SD) at 174 nt (S) to an intronic *Eco*RI (R) site at 193 nt (U). The input probe was 386 nt in length. The probe used in Fig. 1*b*, lanes 5–8 was end-labelled at an insulin gene *Alw*I (W) site using polynucleotide kinase and extends through the insulin splice acceptor (SA) at 110 nt (S), to an intronic *Kpn*I (K) site at 378 nt (U). The input probe was 784 nt in length. The probe used in Fig. 1*b*, lanes 9–18 was end-labelled at a *tat* gene *Xho*II (X) site using Klenow DNA polymerase and extends through the *tat* gene splice donor (SD) at 202 nt (S) to an intronic *Kpn*I (K) site at 506 nt (U). The input probe was 798 nt in length.

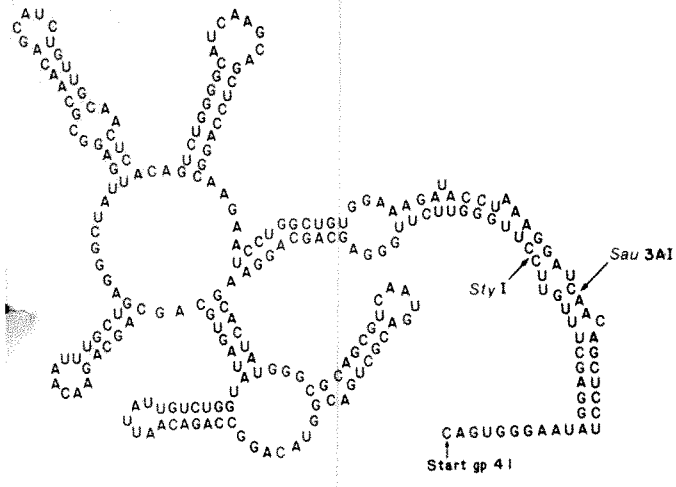


FIG. 2 Predicted secondary structure of the HIV-1 Rev response element. The mutationally defined minimal RRE extends from a 5′ *Sty*I site to a 3′ *Sau*3AI site (piD/G, Fig. 1*a*). This sequence is located immediately 3′ to the start of the gp41 *env* gene-coding region and coincides with the most highly conserved sequence domain within the *env* gene[9]. The RNA stem-loop structure presented is both extremely stable ($\Delta G = -115.1$ kcal/mol^{-1}) and highly significant (segment score in standard deviation units $= -5.29$). In a previous survey, no equivalent predicted secondary structure was observed within the HIV-1 genome[9] and the RRE is therefore likely to be unique. In fact, the RRE would be contained within all known incompletely spliced HIV-1 mRNAs, including the *gag–pol* and *env* mRNAs.

METHODS. The distribution of significant secondary structures in the HIV-1 *env* gene was calculated for window (segment) sizes ranging from 30 to 300 bases by adding two bases to the segment size from 30 to 100 bases and five bases to the segment size from 100 to 300 bases. For each segment size, the calculation was carried out by sliding one base along the *env* gene-coding sequence. The segment scores which measure the statistical significance of the optimal secondary structures were computed[9] using the Cray operating system of a CRAY X-MP/24 computer and the energy rules of Salser[19] and Cech *et al.*[20].

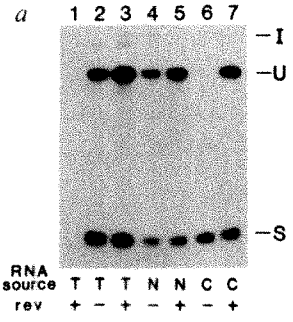
RNA splicing. To examine the alternative hypothesis, that Rev activates the nuclear export of unspliced HIV-1 RNAs[6], we used S_1 nuclease analysis to quantify the effect of Rev on the level of spliced and unspliced *tat* RNA within the total, nuclear and cytoplasmic fractions of cells transfected with pgTAT. As an internal control, these cultures were cotransfected with a genomic rat preproinsulin II gene expression vector[8]. This experiment again showed that unspliced *tat* mRNA contributes only a low percentage of the cytoplasmic steady-state mRNA in the absence of Rev (Fig. 3*a*, lane 6) whereas unspliced cytoplasmic *tat* mRNA predominates in *rev*-expressing cells (Fig. 3*a*, lane 7). In contrast, unspliced *tat* RNA was the predominant *tat* RNA species detected in the nucleus regardless of *rev* co-expression (Fig. 3*a*, lanes 4, 5), indicating that Rev does not significantly affect either the nuclear stability or splicing of HIV-1 transcripts. Interestingly, the level of cytoplasmic unspliced *tat* mRNA expression induced by Rev closely mirrored the high level of unspliced RNA constitutively expressed in the nucleus. This is consistent with the hypothesis that Rev functions to equilibrate the cytoplasm with a pre-existing pool of nuclear viral RNA. The Rev protein also has little effect on the level of unspliced mRNA in total cellular RNA (Fig. 3*a*, lanes 2, 3), suggesting that the nuclear pool of HIV-1 RNA in these transfected cells contributes a surprisingly high percentage of total cellular HIV-1 RNA. A parallel analysis of insulin RNA expression (Fig. 3*b*) reveals that Rev had no detectable effect on the relative expression of spliced and unspliced insulin transcripts. By contrast with the simultaneously expressed *tat* RNA, unspliced insulin RNA was observed at only a very low steady-state level and was excluded from the cytoplasm. The high steady-state level of unspliced nuclear *tat* RNA in these transfected cells indicates that this HIV-1 RNA is poorly recognized by the nuclear splicing machinery. For other retroviruses, sequences have in fact been defined which act constitutively and in *cis* to reduce splicing efficiency[12,13].

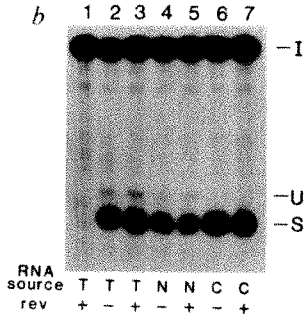
If the Rev protein acts to regulate the export from the nucleus to the cytoplasm of unspliced HIV-1 transcripts then it might also regulate the expression of genomic HIV-1 sequences which are incapable of splicing. In fact, Knight *et al.*[3] have previously shown tht an *env* gene expression vector (pENV160, see Fig. 1*a* legend) which encodes a non-spliceable *env* RNA, remains fully subject to regulation by Rev[3]. Furthermore, the Rev induction of Env protein is not accompanied by a significant increase in total cellular *env* RNA[3]. Our results (Fig. 3*a*) however, suggest, that the latter observation could have resulted from the use of unfractionated RNA in the earlier study. To clarify this, we transfected COS cells with pgTAT or with pENV160 in the presence or absence of Rev. Cytoplasmic RNA from these cultures was then subjected to northern analysis using an *env*-gene-specific probe. As previously reported[6], unspliced cytoplasmic pgTAT RNA was observed only in the presence of Rev (Fig. 3*c*, lanes 3, 4). Remarkably, the cytoplasmic expression of the *env* mRNA encoded by pENV160 was also observed to be fully dependent on *rev* co-expression (Fig. 3*c* lanes 1, 2), showing that the regulation of cytoplasmic HIV-1 RNA expression by Rev occurs independently from the regulation of HIV-1 RNA splicing.

A major question raised by this study is why the high level of unspliced *tat* mRNA detected in the nucleus of transfected cells cannot enter the cytoplasmic compartment in the absence of Rev. One possibility is that the intronic sequences present in pgTAT repress unspliced RNA transport. Indeed, Rosen *et al.*[14] have proposed the existence of four such *cis*-acting repressive (CRS) sequences within the HIV-1 *env* gene. In the absence of *rev* co-expression, the deletion mutant pΔK/Hd, which lacks all four of these proposed repressive sequences, was, however, found to express the same phenotype as the parental pgTAT vector (Fig. 1*c*, lane 5). We therefore favour the alternative hypothesis that incompletely spliced HIV-1 mRNAs are prevented from leaving the nucleus by the same processes which preclude the export from the nucleus of many unspliced or incompletely spliced gene transcripts[13,15–17]. The role of Rev would then be to permit incompletely spliced HIV-1 transcripts to interact with the nuclear RNA export machinery. It remains to be determined if the Rev *trans*-activator mediates the export of unspliced viral RNA from the nucleus by interacting directly with its RNA target sequence or whether Rev instead functions to post-translationally or transcriptionally activate cellular RNA sequence-recognition factors. □

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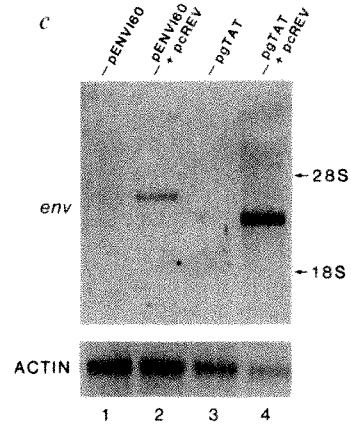


FIG. 3 The *rev* gene product activates the nuclear export of unspliced HIV-1 RNAs. *a*, S_1 nuclease analysis of spliced and unspliced *tat* gene RNA. COS cells were transfected[8] with pgTAT and the genomic rat preproinsulin II gene expression vector pBC12MI in the presence (+) or absence (–) of the *rev* gene expression vector pcREV[6]. Lane 1 contains RNA from a culture transfected with pcREV alone. Total (T) or fractionated cytoplasmic (C) and nuclear (N) RNA was prepared at 48 h after transfection as previously described (21, 22). 5 μg of RNA was used for the total and cytoplasmic S_1 analyses while 2 μg of RNA was used for the nuclear samples. The input (I) probe is identical to the *tat* probe used in Fig. 1*c*, lanes 9–18 and allows the quantitation of both spliced (S) and unspliced (U) *tat* RNAs. The relative level of unspliced *tat* RNA in each sample was determined by densitometry using an LKB soft laser scanner and is: lane 2, 0.59; lane 3, 0.71; lane 4, 0.74; lane 5, 0.81; lane 6, 0.07; lane 7, 0.77. *b*, S_1 nuclease analysis of spliced and unspliced insulin gene RNA. The analysis visualized here was performed precisely as described for Fig. 3*a*, using the same RNA samples, except that the insulin-gene-specific S_1 probe described for Fig. 1*c*, lanes 1–4 was used. This input probe (I) permits the quantitation of both spliced (S) and unspliced (U) insulin RNA. *c*, Northern analysis[23] of cytoplasmic *env* gene mRNA. COS cell cultures were transfected[6] with pgTAT or pENV160 in the presence or absence of pcREV. As the *env* gene in pENV160 is under HIV-1 LTR control, the *tat* gene expression vector pcTAT[6] was also included in each transfection. Cytoplasmic RNA was isolated[21,22] at 48 h after transfection and 5-μg aliquots used for each lane. The unspliced RNA encoded by pgTAT is predicted to be 3.0 kilobases (kb) in length, whereas the non-spliceable *env* mRNA encoded by pENV160 has a predicted size of 3.5kb[3,6]. The filter was initially probed (upper panel) with an *env* gene specific probe derived from the *Bgl*II (B2) to *Hin*dIII (Hd) fragment shown in Fig. 1*a*. Subsequently, the filter was rehybridized with an actin probe as a loading control (lower panel).

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Signal transduction through the CD4 receptor involves the activation of the internal membrane tyrosine-protein kinase p56^{lck}

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THE CD4 T-cell surface antigen is an integral membrane glycoprotein of relative molecular mass 55,000 which binds class II major histocompatibility complex (MHC) molecules expressed on antigen presenting cells (APCs). It is thought to stabilize physical interactions between T cells and APCs (for a review, see ref. 1). Evidence is accumulating that suggests that CD4 can transduce an independent signal during T-cell activation[2–4]. It has recently been shown that CD4 expressed on human[5,6] and murine[6] T cells is physically associated with the Src-related tyrosine protein kinase p56^{lck} (refs 7, 8). These results indicate that CD4 can function as a signal transducer and suggest that tyrosine phosphorylation events may be important in CD4-mediated signalling. Here, we present evidence that cross-linking of the CD4 receptor induces a rapid increase in the tyrosine-specific protein kinase activity of p56^{lck} and is associated with the rapid phosphorylation of one of the subunits (ζ) of the T-cell receptor complex on tyrosine residues. These data provide direct evidence for a specific CD4 signal transduction pathway that is mediated through p56^{lck} and suggest that some of the tyrosine phosphorylation events detected during antigen-mediated T-cell activation may result from signalling through this surface molecule.

To test whether binding of ligand to the extracellular domain of CD4 results in an intracellular signal involving alterations of p56^{lck}, we examined the effects of antibody-mediated cross-linking of cell-surface CD4 on the enzymatic activity of p56^{lck} (Fig. 1). Cloned CD4$^+$/CD8$^-$ murine T lymphocytes (C8, see Fig. 1 legend)[9] were incubated with anti-CD4 monoclonal antibody (mAb) GK1.5 (which reacts against the extracellular domain of CD4) and rabbit anti-rat (RAR) immunoglobulin G. The effects of this treatment on the tyrosine kinase activity of p56^{lck} were analysed by immune-complex kinase assays using Lck-specific antisera (Fig. 1*a*). CD4 cross-linking resulted in a rapid (within 30 seconds) and significant (three to fivefold) increase in the tyrosine-specific protein kinase activity of p56^{lck}, as measured by *in vitro* autophosphorylation and phosphorylation of enolase, an exogenous substrate. A parallel Lck immunoblot (Fig. 1*a*, bottom panel) showed that this change is not attributable to an increase in the abundance of p56^{lck}, suggesting that it reflects an increase in 'specific' enzymatic activity. Similar results were obtained with another CD4$^+$ murine T-cell clone (B10; ref. 6) but were not observed in the CD4$^-$ murine T-cell hybridoma 16M9 (ref. 10). The abundance of the Lck protein seemed to diminish after a few minutes of cross-linking (bottom panel, lanes 4 and 5), consistent with our previous observations[6].

The phosphorylated species migrating at about 64,000 (64K) on this gel appears to result from a non-specific immunoprecipitation (our unpublished data). Indeed, using lysis conditions that preserve the enzymatic activity of Lck as well as its interaction with CD4, another protein, p64, can be recovered by a variety of polyclonal and monoclonal antisera (including preimmune sera). Although prominent in the C8 cells, it is not detected in murine thymocytes in which a similar stimulation of p56^{lck} and enolase phosphorylation can be detected after CD4 cross-linking (manuscript in preparation). Preliminary data indicate that p64 may represent a contaminating serine kinase (unpublished data).

Further experiments (Fig. 1*b*) showed that the stimulation of Lck kinase activity depends on the physical cross-linking of CD4 as the enzymatic activity was unchanged by treatment with monovalent (antigen-binding) fragments (Fab) of mAb GK1.5 (Fig. 1*b*, lane 2). The small increase in kinase activity seen with mAb GK1.5 alone (lane 3) is probably a result of a small amount of CD4 cross-linking induced by the bivalent antibody or by aggregates of the monoclonal antibody. The specificity of the induction of Lck kinase activity by cell-surface antigen cross-linking was evaluated (Fig. 1*c*). Cross-linking of CD4 with a different anti-CD4 mAb (H129.19) (Fig. 1*c*, lane 3) also resulted in a rapid activation of p56^{lck}. Cross-linking of other surface molecules however, such as the T-cell antigen receptor (TCR) complex, which can induce T-cell activation (lane 4), Thy1.2 (lane 5) as well as cross-linking antibody directed against CD8 (using an antibody with the same isotype as mAb GK1.5) (lane 6) did not result in any alteration in enzymatic activity or abundance of the Lck protein.

Because not all p56^{lck} molecules expressed in CD4$^+$ T cells are complexed with CD4 (ref. 6), a more accurate estimate of the degree of enzymatic activation of p56^{lck} requires a specific analysis of the population of Lck molecules associated with CD4. Immune-complex kinase assays of CD4 immunoprecipitates (Fig. 2*a*) revealed that CD4 cross-linking induced a six to eightfold increase in Lck kinase activity (Fig. 2*a*, lane 2). Additional experiments established that CD4 cross-linking did not significantly alter the enzymatic activity of the non-CD4 associated Lck kinase (Fig. 2*b*, lane 2).

The only substrate of an activated tyrosine kinase detected during antigen presentation that has been defined is the ζ-subunit of the TCR complex, which is phosphorylated on tyrosine residues within minutes of antigen-mediated T-cell activation[11–13]. The data reported here raise the possibility that signalling through the CD4-Lck pathway may participate in this phosphorylation event. To determine whether CD4 cross-linking could mediate this alteration, the effect of antibody-mediated cross-linking of CD4 on the state of tyrosine phosphorylation of the ζ-subunit of the TCR complex was examined (Fig. 3). After treating CD4$^+$ murine T cells (C8) in various ways, the components of the TCR complex were immunoprecipitated with

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specific anti-ζ-antibodies and their phosphotyrosine content analysed by antiphosphotyrosine immunoblotting. This is a sensitive and specific method for evaluating tyrosine phosphorylation changes of this TCR complex component[11-13]. The results of several experiments demonstrated that cross-linking of CD4 results in a three to fourfold increase in the phosphotyrosine content of the 21K phosphorylated form of the ζ-subunit (Fig. 3*a*, lane 3; data not shown). In these cells, the extent of the detectable increase in ζ-subunit phosphotyrosine content was similar to that induced upon activation with the combination of a stimulatory antibody to the TCR complex (mAb 145-2c11 cross-linked by Fc receptor-bearing B cells (Fc is the crystallizable fragment of an immunoglobulin) (lane 5). A detailed analysis of these modifications showed that the increase in ζ-subunit tyrosine phosphorylation occurs as soon as one minute after CD4 cross-linking, reaching a maximum after 10–15 min (Fig

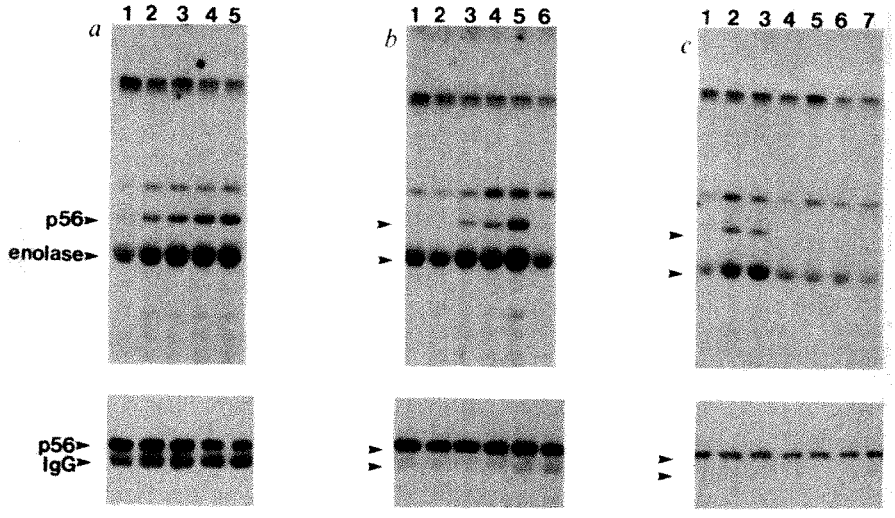


FIG. 1 Effects of surface antigen cross-linking on p56^{lck}. *a*, Time course of the effects of antibody-mediated cross-linking of CD4 on p56^{lck} from a CD4$^+$ murine T-cell clone (C8). Lane 1, untreated control; 2, 30 s; 3, 1 min; 4, 2 min and 5, 3 min. Top panel, autophosphorylation and enolase phosphorylation in *in vitro* kinase reactions. Bottom panel, Lck immunoblot assays. The positions of p56^{lck} (p56), enolase and IgG are indicated. The low level of autophosphorylation seen with Lck molecules from control cells (lane 1) is related to the presence of 10 μm non-radioactive ATP in the enolase phosphorylation reactions, a situation which favours the phosphorylation of the exogenous substrate. Lck autophosphorylation at significant levels (also observed after CD4 cross-linking) can be observed in control cells when the assay mixture contains 1 μM ATP. Similar observations have been made for the c-*src* tyrosine kinase (our unpublished data). Exposure times were: kinase, 6 h; immunoblot, 18 h. *b*, Effects of various treatments with anti-CD4 mAb (mAb GK1.5) on p56^{lck}. Cross-linking was performed for 1 min. Lane 1, untreated control; 2, Fab fragments of mAb GK1.5; 3, mAb GK1.5; 4, mAb GK1.5 with RAR IgG added to the lysate; 5, mAb GK1.5 + RAR IgG and 6, RAR IgG. Top panel, kinase reactions (autophosphorylation and enolase phosphorylation). Bottom panel: Lck immunoblot. Exposure times were as in *a*. *c*, Effects of antibody-mediated cross-linking of various cell-surface antigens on p56^{lck}. Cross-linking was performed for 1 min (similar results were obtained after cross-linking for 5 min, data not shown). Lane 1, untreated control; 2, mAb GK1.5 + RAR IgG; 3, anti-CD4 mAb H129.19 + RAR IgG; 4, anti-CD3 mAb 145-2C11 + rabbit anti-hamster (RAH) IgG; 5, anti-Thy1.2 mAb 30-H12 + RAR IgG; 6, anti-CD8 mAb 2.43 + RAR IgG; and 7, RAR IgG. Top panel, kinase reactions (autophosphorylation and enolase phosphorylation). Bottom panel, Lck immunoblot. Exposure times were kinase, 6 h; immunoblot, 14 h.

METHODS. C8 is an antigen-specific CD4$^+$ murine T-cell hybridoma derived from B6 mice[15]. Cells were grown and prepared as described elsewhere[6,15]. Resting C8 cells (10^7 cells) were incubated on ice with saturating concentrations of various mAbs for 30 min, washed in cold PBS and incubated at room temperature in serum-free DMEM with or without RAR or RAH IgG (10 μg; Organon Teknika) as second-step antibodies. After cross-linking, cells were lysed directly in DMEM by adding an equal volume of modified 2 × TNE buffer (100 mM Tris pH 8.0; 2% NP-40 and 40 mM EDTA, pH 8.0) containing 200 μM sodium orthovanadate, 100 mM sodium fluoride and the protease inhibitors leupeptin and aprotinin (each at a concentration of 20 μg ml^{-1}). Similar results were obtained if the cells were extensively washed in cold PBS before lysis (data not shown). After clarification of the detergent lysate by centrifugation (10,000*g* for 5 min at 4 °C in an Eppendorf microfuge, this centrifugation does not alter the recovery of p56^{lck}, at least within the first few minutes of cross-linking (see Fig. 1)), Lck proteins were immunoprecipitated from equivalent amounts of cellular proteins (50 μg) as described[16]. Immune-complex kinase assays were performed on washed immunoprecipitates in the presence of 10 μM ATP and rabbit muscle enolase, the model substrate[16]. The reactions were stopped by the addition of sample buffer and the products of these reactions resolved by 8% SDS-PAGE[16]. The Lck immunoblot of parallel immunoprecipitates was performed as described[8]. All monoclonal antibodies have been previously described[6] and were used as ammonium sulphate precipitation of ascites. Fab fragments of mAb GK1.5 were kindly provided by Ada Kruisbeek, NCI. We quantitated the changes described in the text by cutting bands from the gel or nitrocellulose and counting in a γ-counter or a liquid scintillation counter.

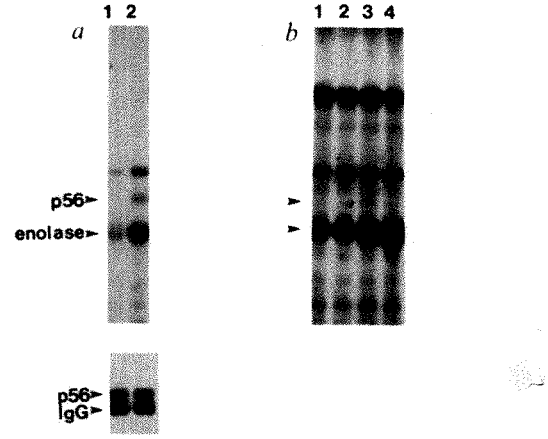


FIG. 2 Effects of CD4 cross-linking on the CD4-associated and non-CD4 associated p56^{lck}. *a*, Effect of CD4 cross-linking on activity of the CD4-associated Lck kinase. Lane 1 untreated control; and 2, mAb GK 1.5 + RAR IgG. Top panel, kinase assays; bottom panel, Lck immunoblot. The positions of p56^{lck}, enolase and IgG are indicated. Exposure times were: kinase, 6 hours; immunoblot, 18 hours (this experiment was done in parallel with that in Fig. 1*a*. *b*, Depletion experiments. Lanes 1 and 2, preclearing with anti-CD4 mAb GK 1.5; 3 and 4, preclearing with anti-Thy1.2 mAb 30-H12. Lanes 1 and 3, untreated cells; 2 and 4, after CD4 cross-linking. The restriction of Lck kinase induction to CD4-associated Lck molecules was demonstrated in a similar way by examining p56^{lck} autophosphorylation in the presence of 1 μM ATP (our unpublished data). The high level of background seen in these assays seems to be related to the extensive manipulations of the cellular lysates. Exposure time was 24 h.

METHODS. C8 cells were treated with a CD4 cross-linking antibody regimen (mAb GK 1.5 + RAR IgG) for 1 min and lysed as described in the Fig. 1 legend. CD4 was immunoprecipitated from equivalent amounts of cellular proteins (50 μg) by a combination of mAb GK 1.5, RAR IgG and *Staphylococcus aureus* protein A (this regimen was shown to recover all CD4-associated Lck, even after antibody-mediated cross-linking of surface CD4 with mAb GK 1.5 and RAR IgG, our unpublished data). For depletion experiments, the cellular lysate was first incubated at 4 °C for 2 h in the presence of the anti-CD4 mAb GK 1.5 or the anti-Thy1.2 mAb 30-H12 and then for 1 h in the presence of *Staph. aureus* protein A coupled to RAR IgG. After removing the immune complexes by centrifugation, the residual Lck proteins in the supernatant were collected by the addition of Lck-specific antisera[16]. Immune-complex kinase assays and immunoblot were performed as previously described[16].

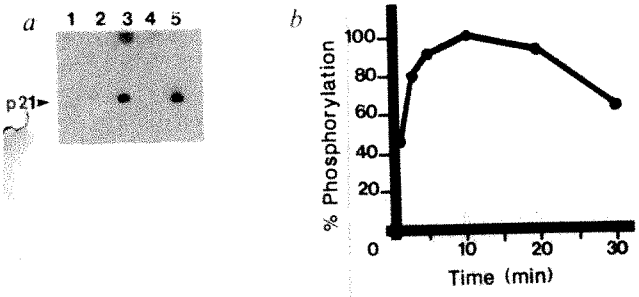


FIG. 3 Effect of CD4 cross-linking on the phosphorylation of ζ-subunit of the TCR complex. *a*, Effects of various antibody regimens. Lane 1, untreated control; 2, mAb GK1.5; 3, mAb GK1.5 and RAR IgG; 4, RAR IgG alone and 5, C8 cells incubated with the B-cell tumour LB and the anti-CD3 (epsilon) mAb 145-2C11 (ref. 6). Exposure was for 72 h. *b*, Time course. C8 cells were incubated with mAb GK1.5, washed and incubated at 37 °C as in Fig. 3*a* for the time indicated with RAR IgG. The level of ζ-chain phosphorylation was analysed with an LKB densitometer and is expressed here as a percentage of maximum-ζ-subunit phosphorylation. The results presented here are derived from the combined analysis of two separate experiments. In both cases, baseline p21(ζ) tyrosine phosphorylation was higher than that seen in the experiment represented in Fig. 3*a*. The basis for this variability is unclear. METHODS. C8 cells were subjected to CD4 cross-linking as described in Fig. 1 or cultured at a 1:1 ratio with the B-cell tumour LB 27.4 plus mAb 145-2C11 culture supernatant at 37 °C (used as T-cell activating stimuli). Cells were lysed as described and immunoprecipitated with an anti-peptide antibody recognizing the TCR-ζ-chain[13]. Electrophoresis and immunoblotting with affinity-purified anti-phosphotyrosine antibodies was performed as previously described[11–13]. Immunoprecipitation of p21 was specific for the ζ-subunit (control sera failed to precipitate this structure; data not shown). The specificity of the anti-phosphotyrosine antibodies used in these assays has previously been described[11–13].

3*b*). Similar increases in ζ-subunit tyrosine phosphorylation were also detected after CD4 cross-linking of another $CD4^+$ murine T-cell clone as well as a $CD4^+$ T-cell tumour line, whereas no effect was seen upon similar treatment of a $CD4^-$ T-cell hybridoma (L.E.S., unpublished data). It is unlikely that the tyrosine phosphorylation of the ζ-subunit reported here represents a non-specific reaction to cell-surface antigen cross-linking, as cross-linking of other surface molecules (such as LFA-1 and H-2) does not result in any significant alteration in the phosphorylation state of the ζ-subunit (L.E.S., unpublished data).

Our results indicate that the interaction of CD4 with its ligand results in a biochemical signal involving the rapid activation of $p56^{lck}$, providing direct evidence for the existence of a signalling pathway associated with CD4. These results also support the view that the CD4–Lck complex functionally mimics growth factor receptors with associated tyrosine kinase function. Although the exact mechanism of enzymatic activation of $p56^{lck}$ remains to be established, preliminary data demonstrate that CD4 cross-linking is associated with a rapid and marked increase in the phosphorylation of Tyr 394 (major site of *in vitro* phosphorylation) and Tyr 505 (major site of *in vivo* phosphorylation) of $p56^{lck}$ (our unpublished data). Interestingly, the phosphorylation state of the analogous residues of $pp60^{c\text{-}src}$ has a significant effect on its tyrosine kinase activity (for a review, see ref. 14). □

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Association of dystrophin and an integral membrane glycoprotein

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DUCHENNE muscular dystrophy (DMD) is caused by a defective gene found on the X-chromosome[1]. Dystrophin is encoded by the DMD gene and represents about 0.002% of total muscle protein[2]. Immunochemical studies have shown that dystrophin is localized to the sarcolemma in normal muscle but is absent in muscle from DMD patients[3–7]. Many features of the predicted primary structure of dystrophin are shared with membrane cytoskeletal proteins[8], but the precise function of dystrophin in muscle is unknown. Here we report the first isolation of dystrophin from digitonin-solubilized skeletal muscle membranes using wheat germ agglutinin (WGA)-Sepharose. We find that dystrophin is not a glycoprotein but binds to WGA-Sepharose because of its tight association with a WGA-binding glycoprotein. The association of dystrophin with this glycoprotein is disrupted by agents that dissociate cytoskeletal proteins from membranes. We conclude that dystrophin is linked to an integral membrane glycoprotein in the sarcolemma. Our results indicate that the function of dystrophin could be to link this glycoprotein to the underlying cytoskeleton and thus help either to preserve membrane stability or to keep the glycoprotein non-uniformly distributed in the sarcolemma.

The specific binding of dystrophin to wheat germ agglutinin (WGA)-Sepharose is shown in Fig. 1 (*a–d*). Dystrophin was solubilized from rabbit skeletal muscle membranes with 0.5 M NaCl and 1% digitonin (Fig. 1*a* and *b*, lane 1). The solubilized membranes were first passed through an antibody affinity column to remove the ryanodine receptor[9] (relative molecular mass, M_r, 450,000). This step is important because of the similar apparent mobilities on SDS-polyacrylamide gels of dystrophin and a major proteolytic fragment of the ryanodine receptor[9]. The flow-through of the ryanodine-receptor affinity column was then applied to a WGA-Sepharose 4B column. Immunoblot-analysis of solubilized membranes and of solubilized membranes that had been repeatedly passed through a WGA-Sepharose column showed that dystrophin was completely removed from solubilized membranes by WGA-Sepharose (Fig. 1*b*, lane 2). Coomassie-blue staining of both samples run on SDS-polyacrylamide gels (Fig. 1*a*) indicated that there had been no change in the protein components because most of the proteins in the rabbit skeletal muscle membrane preparation are not WGA-binding glycoproteins. The protein adsorbed to WGA-Sepharose was also analysed by SDS-PAGE and immunoblotting (Fig. 1*c* and *d*). In the absence of *N*-acetyl-D-glucosamine (NAG), the α_1 and α_2 subunits of the dihydropyridine (DHP) receptor and a protein of M_r 400K were the main proteins bound to WGA-

Sepharose (Fig. 1*c*, lane 1). The high-molecular weight protein was identified as dystrophin by immunoblotting (Fig. 1*d*, lane 1). The specificity of the absorption of dystrophin to WGA-Sepharose was tested by repeating the experiment in the presence of NAG. Only myosin ($M_r \approx 205$K) and the ($Ca^{2+}+Mg^{2+}$) ATPase ($M_r \approx 105$K) bound nonspecifically to WGA-Sepharose in the presence of NAG (Fig. 1*c*, lane 2). Our results therefore demonstrate that dystrophin specifically binds to WGA-Sepharose following solubilization in digitonin. The specific elution of dystrophin from WGA-Sepharose by NAG is shown in Fig. 1*e* and *f*; the yield of NAG-eluted material is ~5 mg from 800 mg starting membranes. The peak protein fractions that are eluted with NAG are enriched in dystrophin and the DHP receptor, as shown by Coomassie-blue staining and immunoblotting of SDS-polyacrylamide gels. Dystrophin makes up about 5% of the total NAG-eluted protein as estimated from integration of densitometer scans of the gels.

The fact that dystrophin could be isolated using WGA-Sepharose following detergent solubilization of skeletal muscle membranes indicated to us that dystrophin might be associated with the DHP receptor, or that it could be a glycoprotein. To investigate the possible association of dystrophin with the DHP receptor, we combined and fractionated NAG-eluted fractions from WGA-Sepharose using DEAE-cellulose. Elution of the DEAE-cellulose column with a linear gradient of NaCl indicated that most of the DHP receptor eluted in a broad pattern from 50–110 mM NaCl, whereas dystrophin eluted at 110–300 mM NaCl. Consequently we developed a step gradient of NaCl to separate the DHP receptor from dystrophin more effectively (Fig. 2). The first four fractions are enriched in DHP receptor and do not contain dystrophin (as determined by Coomassie blue-stained gels and immunoblots; Fig. 2*a* and *b*, lanes 2–5). The final fraction is enriched in dystrophin (corresponding to 30–40% of the protein by densitometry) and is essentially depleted of DHP receptor (Fig. 2*a* and *b*, lane 6). Proteins co-eluting with dystrophin migrated on Coomassie blue-stained gels at positions corresponding to M_rs of 325K, 250K, 225K, 172K, 162K, 143K, 57K, 47K, 39K and 31K; the mobility of dystrophin indicated that its M_r was ~360K. Immunoblotting after each step in the preparation showed that dystrophin was isolated intact and not degraded. Thus the decrease in apparent M_r of isolated dystrophin when compared with the value of 427K predicted from the complementary DNA sequence[8] may result from proteolytic processing after synthesis or simply from the difficulty in measuring high molecular weight proteins accurately on SDS gels.

To investigate whether dystrophin is a glycoprotein, we stained protein blots of DEAE-isolated dystrophin and purified DHP receptor with ^{125}I-labelled lectins. ^{125}I-labelled WGA specifically stained the α_2 subunit of the DHP receptor (Fig. 3,

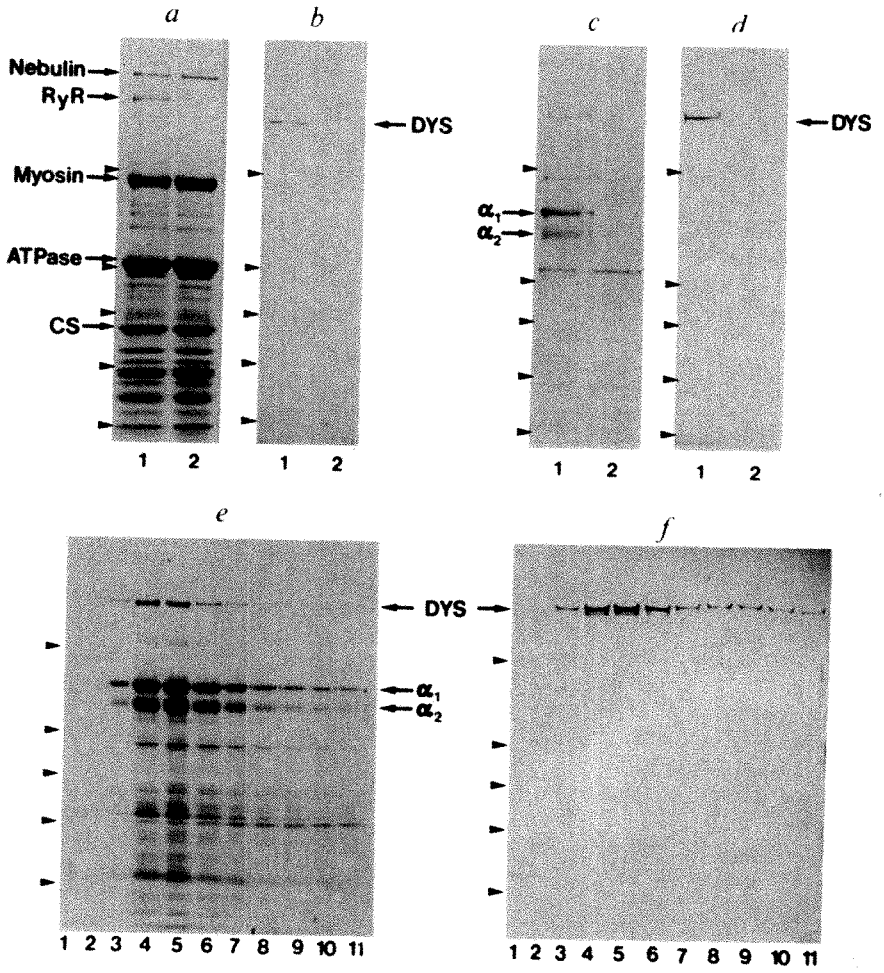


FIG. 1 Specific binding of dystrophin to WGA-Sepharose (*a–d*) and elution with *N*-acetyl-D-glucosamine (*e–f*). Coomassie blue-stained gels (*a*, *c* and *e*) and immunoblots of identical gels stained with sheep polyclonal anti-dystrophin antibodies (*b*, *d* and *f*) are shown. *a* and *b*, Lane 1, digitonin-solubilized rabbit skeletal-muscle membranes (75 μg); lane 2, column flow-through from four independent WGA-Sepharose columns (75 μg). Arrows indicate the positions of nebulin, ~700K; ryanodine receptor/Ca^{2+}-release channel, ~450K (RyR); myosin, 205K; ($Ca^{2+}+Mg^{2+}$)ATPase, 105K (ATPase); and calsequestrin, 63K (CS). *c* and *d*, WGA-Sepharose beads (50 μl) incubated with solubilized membranes in the absence (lane 1) or presence (lane 2) of *N*-acetyl-D-glucosamine. *e* and *f*, Lanes 1–11 are 100 μl aliquots of NAG-eluted WGA-Sepharose column fractions. Arrows indicate the positions of dystrophin (DYS) in panels *b*, *d*, *e* and *f* and the α_1 and α_2 subunits of the DHP receptor in panels *c* and *e*. For all panels, arrowheads indicate the positions of the molecular weight standards: from top to bottom their M_r values ($\times 10^{-3}$) are 208, 100, 68, 43 and 25.

METHODS. For *a* and *b*, rabbit skeletal muscle membranes[9] (800 mg) were solubilized with a solution of 1% digitonin and 0.5 M NaCl containing protease inhibitors at a protein concentration of 4 mg ml^{-1}, as previously described[9], then passaged through XA7-Sepharose for removal of ryanodine receptor[8] and through four different columns of WGA-Sepharose (15 ml each). For *c* and *d*, membranes (2 mg) were solubilized and passed through XA7-Sepharose as already described, then applied to 50 μl WGA-Sepharose in the absence or presence of 0.3 M NAG. After incubation the WGA-Sepharose beads were washed extensively and subjected to SDS-PAGE. For *e* and *f*, digitonin-solubilized membranes (800 mg) were applied to XA7-Sepharose and circulated overnight on 15 ml WGA-Sepharose. After extensive washing, the WGA-Sepharose column was eluted with five column volumes of 0.3 M NAG and 2.5 ml fractions were collected. Yields of 3 to 5 mg from 800 mg solubilized membranes were typical. In all experiments, WGA-Sepharose buffers contained 50 mM Tris-HCl, pH 7.4, 0.75 mM benzamidine and 0.1 mM phenylmethylsulphonyl fluoride. Samples were fractionated on 3–12% gradient SDS-polyacrylamide gels[17] and either stained with Coomassie blue or transferred to nitrocellulose paper[18] and stained with either sheep polyclonal anti-dystrophin antibodies[19] or anti-α_1 and anti-α_2 antibodies[20,21] for identification of the α_1 and α_2 subunits of the DHP receptor (not shown). Gel lanes were scanned using a Hoefer GS 300 scanning densitometer and analysed using GS-360 data analysis software. Protein concentrations were determined as previously described[22].

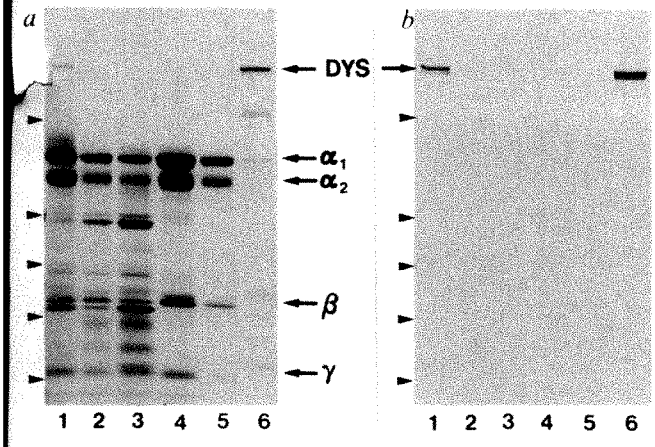


FIG. 2 Isolation of dystrophin using DEAE-cellulose. A Coomassie blue-stained gel (*a*) and an immunoblot of an identical gel stained with sheep polyclonal anti-dystrophin antibodies (*b*) are shown. Lane 1, 10 μg NAG-eluted sample (Fig. 1*e*, lane 5); lane 2, 13 μg fraction 1 from the 50 mM NaCl wash from the DEAE column; lane 3, 15 μg fraction 2 from the DEAE-column 50 mM NaCl wash; lane 4, 25 μg fraction 1 from the DEAE-column 100 mM NaCl wash; lane 5, 11 μg fraction 2 from the DEAE-column 100 mM NaCl wash; lane 6, 8 μg fraction 1 from the DEAE-column 250 mM NaCl wash. Arrows indicate the positions of dystrophin (DYS) and the α_1, α_2, β and γ subunits of the DHP receptor. Molecular weight standards (arrowheads) are the same as those used for Fig. 1.

METHODS. Eluted fractions from WGA-Sepharose (Fig. 1*e*) were applied to 2 ml packed DEAE-Cellulose equilibrated in buffer A (0.1% digitonin, 50 mM Tris-HCl, pH 7.4, 0.75 mM benzamidine and 0.1 mM phenylmethylsulphonyl fluoride). The DEAE-column was washed with 10 ml buffer A, followed by buffer A containing 50 mM NaCl (50 ml), 100 mM NaCl (200 ml), 110 mM NaCl (100 ml) and 250 mM NaCl (25 ml). Fractions (2.5 ml) were collected for all washes. Analysis of the 110 mM NaCl wash indicated a protein pattern similar to that in lanes 4 and 5, but much less intense (not shown). Washing of the DEAE-column with NaCl concentrations between 110 mM NaCl and 250 mM revealed a protein pattern similar to that in lane 6 and did not preferentially remove any protein (not shown). Approximately 300 μg isolated dystrophin (lane 6) was obtained from 700 mg solubilized membranes. SDS-PAGE, immunoblot analysis and densitometer scanning are described in the legend to Fig. 1.

lane 1) but did not stain dystrophin (Fig. 3, lane 2). Concanavalin A (con A) labelled with ^{125}I was also used to stain blots and together with results of endoglycosidase H digests confirmed that dystrophin is not a glycoprotein (data not shown). So we conclude that dystrophin must be retained by WGA-Sepharose as a result of an association with a glyco-mediator which survives solubilization of the membranes. The isolated dystrophin also binds specifically to con A-Sepharose (not shown). As glycoproteins are the only molecules that will specifically bind both to WGA-Sepharose and con A-Sepharose, the mediator must be a glycoprotein. We also show (Fig. 3) that the dystrophin preparation contains several glycoproteins ranging from 40K to 500K, but no glycolipids. Therefore the mediator of the interaction between dystrophin and the lectin columns must be a glycoprotein containing both WGA and con A binding sites. Also, as the detergent concentration necessary to solubilize dystrophin is almost identical to that required to solubilize the ryanodine and DHP receptors (both integral membrane proteins), then the mediator must also be an integral membrane glycoprotein.

To investigate this association of dystrophin with the integral membrane glycoprotein, we attempted to dissociate the dystrophin-glycoprotein complex with treatments known to disrupt the interaction between cytoskeletal proteins and membranes[10-12], and then monitored the binding of dystrophin to WGA-Sepharose. We used NAG-eluted dystrophin because we needed large amounts of protein for Coomassie-blue staining and because it contained the DHP receptor, which is a complex of several polypeptides with a major glycoprotein[13]. Figure 4 shows that NAG-eluted dystrophin rebinds completely to WGA-Sepharose after removal of NAG (lane 1). Thus, dystrophin retains its association with the WGA-binding glycoprotein after isolation and dialysis to remove NAG. When the NAG-eluted sample is treated with 1% SDS, followed by dilution to 0.1% SDS and removal of NAG by dialysis, dystrophin no longer binds to WGA-Sepharose (Fig. 4*a* and *b*, lane 2), even though the α_2 subunit of the DHP receptor (a WGA-binding glycoprotein) still binds to WGA-Sepharose (Fig. 4*c* and *d*, lane 2). Thus, SDS is able to dissociate the dystrophin–glycoprotein complex and the free dystrophin does not bind to WGA-Sepharose.

Membrane cytoskeletal protein interactions are known to be sensitive to molar concentrations of potassium iodide[10,11]. We tested the effect of KI on the interaction of dystrophin with the WGA-binding glycoprotein by treating the NAG-eluted sample with 2 M KI. After dilution of KI to 0.5 M and removal of NAG by dialysis, the sample was applied to WGA-Sepharose. In Fig. 4, lane 3 it is shown that KI dissociates the dystrophin–glycoprotein complex and that KI-treated dystrophin no longer binds to WGA-Sepharose, even though the α_2 subunit of the

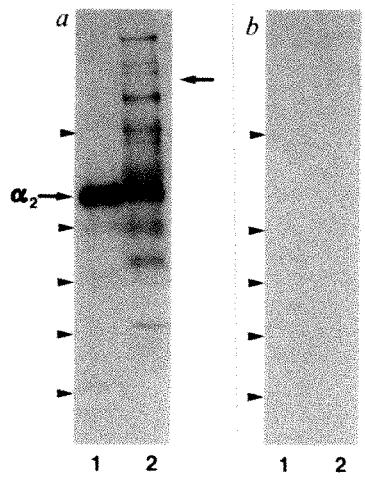


FIG. 3 Glycoprotein analysis of purified DHP receptor and isolated dystrophin using ^{125}I-labelled WGA. Autoradiographs of immunoblots incubated with ^{125}I-labelled WGA in the absence (*a*) or presence (*b*) of 0.3 M NAG are shown. Lane 1, 7 μg purified DHP receptor (Fig. 2, lane 4); lane 2, 7 μg isolated dystrophin (Fig. 2, lane 6). The position of the α_2 subunit of the DHP receptor is indicated by the arrow (α_2). The arrow on the right in panel *a* indicates the position of dystrophin determined by staining the blot with sheep polyclonal anti-dystrophin antibodies (after staining with ^{125}I-labelled WGA) and then overlaying the autoradiograph on the blot. Note that dystrophin does not stain with ^{125}I-labelled WGA. Molecular weight standards (arrowheads) are the same as those used for Fig. 1.

METHODS. DHP receptor (Fig. 2, lane 4) or isolated dystrophin (Fig. 2, lane 6) were separated on 3–12% SDS-polyacrylamide gels and transferred to nitrocellulose paper. Blots were blocked in PBS-Tween (50 mM NaH_2PO_4, 0.9% NaCl, 0.05% Tween-20) and incubated for 1 h at 25 °C with 250,000 c.p.m. ^{125}I-labelled WGA per ml PBS-Tween either with or without 0.3 M NAG. Transfers were washed extensively with PBS-Tween then with PBS, then dried and exposed to Kodak-XAR film using intensifying screens (Dupont). Exposures were for three days at 25 °C. No additional bands appeared after longer exposure. Identical results were obtained using ^{125}I-labelled con A (not shown). SDS-PAGE and immunoblot analysis are described in the legend to Fig. 1.

DHP receptor still binds to WGA-Sepharose. Similar results were obtained with con A-Sepharose (not shown). ^{125}I-labelled WGA staining of the material in the void volume from the WGA-Sepharose beads and of the NAG-eluted material (not shown) indicates that all WGA-binding glycoproteins bound to WGA-Sepharose after treatment with SDS or KI. So, dystrophin binds to WGA-Sepharose as a result of its tight association with a WGA-binding glycoprotein and this association is sensitive to SDS and KI.

Here we have reported the first isolation of dystrophin from skeletal muscle membranes: as dystrophin is present in very low concentrations in muscle, a procedure representing a 17,000-fold purification of dystrophin from whole muscle is an important advance. Previously dystrophin could only be studied indirectly, for example by using immunological probes for the protein. We have also demonstrated that dystrophin is tightly associated with an integral membrane glycoprotein and that this association i disrupted by agents that dissociate cytoskeletal proteins fron membranes. The similarities between dystrophin and th cytoskeletal proteins α-actinin and spectrin[8] further indicat that the function of dystrophin could be to link an integra membrane glycoprotein to the underlying cytoskeleton. Thi association of dystrophin with a WGA-binding glycoprotein ha been found in membranes from rabbit and rat cardiac muscl and skeletal muscle from guinea pig, dog, mouse and humar (results not shown). We conclude that the localization o dystrophin to the cytoplasmic face of the sarcolemma result from a tight association with an integral sarcolemma membran glycoprotein and we propose that this glycoprotein serves as a 'dystrophin receptor' which links dystrophin to the sarcolemma membrane. Although we have not yet identified this 'receptor', the results shown in Fig. 3 indicate that it could be one of the glycoproteins migrating with M_r 480K, 400K, 362K, 300K, 226K, 205K, 133K, 100K, 75K, 62K, 47K or 37K.

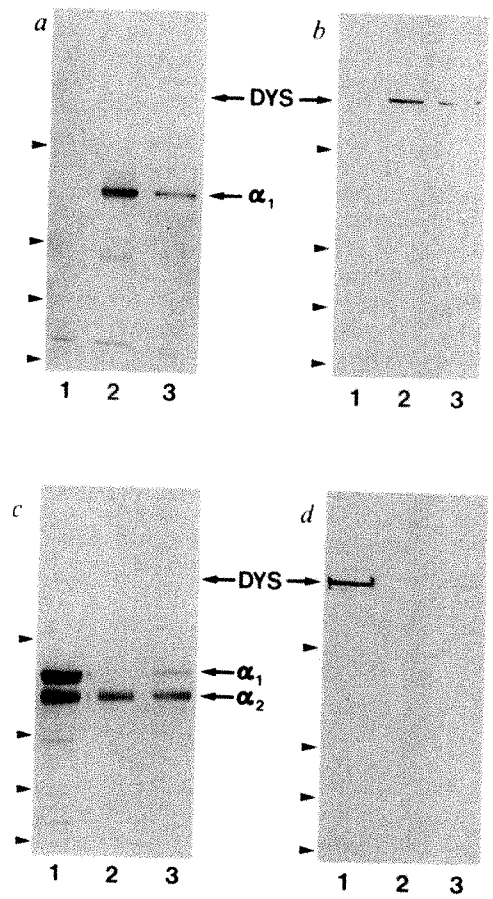


FIG. 4 Dissociation of dystrophin and membrane-associated glycoproteins using SDS and KI. Shown are Coomassie Blue-stained gels (*a* and *c*) and immunoblots of identical gels stained with sheep polyclonal anti-dystrophin antibodies (*b* and *d*). Lane 1, no treatment; lane 2, 1% SDS treatment; lane 3, 2 M KI treatment. In *a* and *b*, supernatants (100 μl) from the WGA-Sepharose beads are analysed. In *c* and *d*, NAG-eluates (100 μl) from the WGA-Sepharose beads used in *a* and *b* are analysed. The positions of dystrophin (DYS) and the α_1 and α_2 (*c* and *d* only) subunits of the DHP receptor are indicated by arrows. Molecular weight standards (arrowheads) are the same as those used for Fig. 1.
METHODS. 100 μl (20 μg) NAG-eluted sample from WGA-Sepharose (Fig. 1*e*, lane 5) was treated for 30 min at 25 °C either alone or with 1% SDS or 2 M KI. Samples were dialysed overnight in 50 mM Tris-HCl, pH 7.4, 0.5 M NaCl, 0.75 mM benzamidine and 0.1 mM phenylmethylsulphonyl fluoride-containing buffer only for the no-treatment sample, 1% SDS for the SDS-treated sample, or 0.5 M KI for the KI-treated sample, and then applied to 50 μl WGA-Sepharose prewashed either with buffer only, 0.1% SDS or 0.5 M KI. The samples were incubated for 2 h at 25 °C with agitation, then pelleted. Following extensive washing with the appropriate buffer (buffer only, 0.1% SDS or 0.5 M KI), the WGA-Sepharose beads were eluted with 0.3 M NAG. SDS-PAGE and immunoblot analysis are described in the legend to Fig. 1.

The dystrophin–glycoprotein complex may have an important structural and/or functional role in muscle: it could contribute to stabilization of the membrane or of a non-uniform distribution of a membrane glycoprotein, or it could link dystrophin to the extracellular matrix. We believe that the low concentration of dystrophin itself favours a role in maintaining the heterogeneous membrane arrangement of a scarce glycoprotein, possibly an ion channel or cell-surface receptor. The association of dystrophin with a sarcolemma glycoprotein is comparable to the association of ankyrin with red-cell or brain membranes[10–12] in that molar concentrations of KI can disrupt the complex. Recently ankyrin has been shown to be associated with the $(Na^+ + K^+)$ATPase in kidney[14] and with the Na^+ channel in the brain[15]; these associations with ankyrin were proposed to be responsible for the non-uniform distribution of these proteins in epithelial cells or neuronal cells respectively. Dystrophin may have a similar role in muscle and could be responsible for a non-uniform distribution of a membrane glycoprotein in the sarcolemma membrane.

The absence of dystrophin in DMD muscle may result in the displacement or dysfunction of the membrane glycoprotein that is normally associated with dystrophin. The lack of the dystrophin–glycoprotein complex may weaken sarcolemma membrane integrity and this may be critical for fast muscle fibres, which appear to be the first site of degeneration[16]. It remains to identify the 'dystrophin receptor' in the sarcolemma membrane and to determine the role of dystrophin in the function of the membrane glycoprotein. □

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A *Drosophila* tissue polarity locus encodes a protein containing seven potential transmembrane domains

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THE function of the *frizzled* (*fz*) locus in *Drosophilia melanogaster* is required to coordinate the cytoskeletons of epidermal cells to produce a parallel array of cuticular hairs and bristles (for example on the wild-type wing all hairs point towards the distal tip)[1,2]. In *fz* mutants it is not the structure of individual hairs and bristles that is altered, but their orientation with respect to their neighbours and the organism as a whole. Mitotic clone analysis[3] indicates that *fz* has two functions in the developing wing. It is required for the proximal-distal transmission of an intercellular polarity signal, a process that is expected to be at least partly extracellular. It is also required for cells to respond to the polarity signal, which is expected to be a cytoplasmic function. The *fz* locus could encode either one bifunctional or two single-function proteins. We report here that, in pupae, *fz* produces a messenger RNA that encodes a protein with seven putative transmembrane domains. Thus, the Fz protein should contain both extracellular and cytoplasmic domains, which could function in the transmission and interpretation of polarity information, respectively. This is the first reported sequence for the protein product of a tissue polarity gene.

The *fz* locus was cloned by transposon tagging[4,5] and *fz* complementary DNA clones isolated[6] (the detailed molecular analysis of the *fz* locus will be presented elsewhere). A single 4 kilobase (kb) hybridization band is seen when northern blots of poly(A)$^+$ pupal RNA are probed with cDNA sequences, suggesting that *fz* encodes a single bifunctional protein.

The 2,466-base pair (bp) sequence of a *fz* cDNA clone is shown in Fig. 1. There is a single complete long open reading frame of 581 amino acids. The Fz protein sequence has several interesting features. Most notably, hydrophobicity analysis[7,8] reveals seven strongly hydrophobic stretches (regions 1 to 7 in Fig. 2), which are typical of transmembrane domains of integral membrane proteins. As *fz* is required for the transmission of an intercellular polarity signal, we suggest that the protein product is located in the plasma membrane of epidermal cells. The N terminus of the Fz protein probably encodes a signal sequence for membrane transport, as typical n (positively charged N-terminal, Arg 3), h (hydrophobic, Ile 5–Ile 13), and c (polar C-terminal, Gln 14–Asp 25) regions are present[9]. In addition, a potential cleavage site for the signal peptidase is present at Ala 26. This suggests that the terminus of Fz is extracellular. If each of the seven hydrophobic regions (Fig. 2) are trans-

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GAA TTC GAC TCC GTG TTT GTT CAG TTA TTT TTT TGC GCG GTT TTC GAA AAA GTG AAA GAA  60
ATA AGG AAA TTC AAA AAG CGG GGG AGG CAT AAA TGA TAT ACA GCT AAC GGT ATT TAT GTG 120
CAT ACA GCA AGA TAA AAA TGT GGC TGC TAG AAA AGT AAA GCC CGG TAC TTT GCC CGA TAA 180
AAG CCA ACG CCA CTA AGT CCT AAA GAC TCC CAA AAA TTA AAT TTT GAC ACA ATA ATA TTT 240
TGC CAA CAA CAA ATA GCA AAG TCG AAT TCA AAC GGT TAA ACA AAA TTA AAG CCT GAA AAT 300
AAC AAA AAG GTC TAA ACA AAA TAA AGA GCA AAA GCA AAC GCA AAT AAG AGA ATA CAG AGA 360
AAT ATT TAT TAT TTG CTT ATA AGG CCA AAC TAA ATA TTT GCC GCC GGC AAA TGT TTA AAA 420
GGT GTT AAG TGT TTC GTG GGA GGG AAG TTT TTA AAC AGG GAA AAT AAA TCA AAT AAG TAT 480
TCA ATG GTG TAT AAG TGA GTG CAA GTA GAT GAG CAA CAT GTG TGT GTT TTA AAG TGT TTA 540
AAG AGA TCG AGA AAA AGC CCC AAA AGT TCT TTG ACA GCG ATA TCG AAA AAT TCC AAA ATG 600
                                                                        Met   1

TGG CGT CAA ATC CTG TTT ATT TTA CCC ACC CTG ATA CAG GGG GTC CAG CGC TAC GAT CAG 660
Trp Arg Gln Ile Leu Phe Ile Leu Pro Thr Leu Ile Gln Gly Val Gln Arg Tyr Asp Gln  21

AGC CCC CTC GAT GCG AGT CCG TAT TAT CGC AGC GGC GGG GGA TTA ATG GCC AGT TCT GGG 720
Ser Pro Leu Asp Ala Ser Pro Tyr Tyr Arg Ser Gly Gly Gly Leu Met Ala Ser Ser Gly  41

ACC GAA CTA GAT GGA CTG CCA CAT CAC AAT CGC TGT GAA CCC ATC ACC ATA TCG ATC TGC 780
Thr Glu Leu Asp Gly Leu Pro His His Asn Arg Cys Glu Pro Ile Thr Ile Ser Ile Cys  61

AAG AAT ATA CCA TAT AAC ATG ACC ATT ATG CCA AAT CTT ATT GGC CAT ACC AAG CAG GAG 840
Lys Asn Ile Pro Tyr Asn Met Thr Ile Met Pro Asn Leu Ile Gly His Thr Lys Gln Glu  81
                                *
GAG GCG GGT CTG GAG GTC CAT CAG TTT GCT CCG CTC GTG AAG ATC GGC TGC AGT GAT GAC 900
Glu Ala Gly Leu Glu Val His Gln Phe Ala Pro Leu Val Lys Ile Gly Cys Ser Asp Asp 101

CTC CAG TTG TTC CTC TGT TCC CTG TAC GTT CCG GTC TGC ACA ATT TTG GAG CGA CCC ATT 960
Leu Gln Leu Phe Leu Cys Ser Leu Tyr Val Pro Val Cys Thr Ile Leu Glu Arg Pro Ile 121

CCG CCT TGT CGA TCT CTG TGC GAA TCG GCG CGG GTA TGT GAA AAG TTA ATG AAA ACC TAC 1020
Pro Pro Cys Arg Ser Leu Cys Glu Ser Ala Arg Val Cys Glu Lys Leu Met Lys Thr Tyr  141

AAC TTT AAC TGG CCG GAA AAT CTG GAG TGC TCC AAA TTT CCC GTC CAT GGA GGC GAG GAT 1080
Asn Phe Asn Trp Pro Glu Asn Leu Glu Cys Ser Lys Phe Pro Val His Gly Gly Glu Asp  161

TTG TGC GTG GCG GAG AAT ACC ACA TCA TCG GCC TCC ACG GCG GCC ACG CCC ACA AGG AGT 1140
Leu Cys Val Ala Glu Asn Thr Thr Ser Ser Ala Ser Thr Ala Ala Thr Pro Thr Arg Ser  181
                        *
GTG GCT AAG GTC ACT ACC CGT AAA CAC CAG ACG GGC GTA GAA AGT CCG CAC CGA AAC ATT 1200
Val Ala Lys Val Thr Thr Arg Lys His Gln Thr Gly Val Glu Ser Pro His Arg Asn Ile  201

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GGC GGA AAG GAT CTG CAT GAC TGT GGA GCC CCA TGT CAT GCC ATG TTC TTC CCG GAG AGA 1320
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Glu Arg Thr Val Leu Arg Tyr Trp Val Gly Ser Trp Ala Ala Val Cys Val Ala Ser Cys  261 M1

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Leu Phe Thr Val Leu Thr Phe Leu Ile Asp Ser Ser Arg Phe Arg Tyr Pro Glu Arg Ala  281 M2


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Ile Val Phe Leu Ala Val Cys Tyr Leu Val Val Gly Cys Ala Tyr Val Ala Gly Leu Gly  301

GCG GGC GAC TCT GTG TCG TGC CGC GAA CCA TTT CCG CCG CCC GTC AAA CTC GGC CGC CTG 1560
Ala Gly Asp Ser Val Ser Cys Arg Glu Pro Phe Pro Pro Pro Val Lys Leu Gly Arg Leu  321

CAG ATG ATG TCC ACC ATC ACC CAG GGC CAC CGA CAA ACC ACG TCC TGC ACG GTT TTA TTC 1620
Gln Met Met Ser Thr Ile Thr Gln Gly His Arg Gln Thr Thr Ser Cys Thr Val Leu Phe  341 M3

ATG GCA CTC TAC TTC TGT TGC ATG GCG GCC TTC GCG TGG TGG TCG TGT CTG GCA TTC GCC 1680
Met Ala Leu Tyr Phe Cys Cys Met Ala Ala Phe Ala Trp Trp Ser Cys Leu Ala Phe Ala  361

TGG TTT TTG GCC GCT GGC CTC AAA TGG GGC CAC GAG GCG ATT GAG AAC AAG TCG CAC TTA 1740
Trp Phe Leu Ala Ala Gly Leu Lys Trp Gly His Glu Ala Ile Glu Asn Lys Ser His Leu  381 M4
                                                                *
TTC CAC CTG GTT GCC TGG GCG GTG CCC GCC CTT CAG ACC ATC TCC GTT CTG GCC CTG GCT 1800
Phe His Leu Val Ala Trp Ala Val Pro Ala Leu Gln Thr Ile Ser Val Leu Ala Leu Ala  401

AAA GTT GAA GGT GAC ATC CTT TCT GGC GTT TGT TTC GTG GGT CAG CTG GAC ACG CAC TCC 1860
Lys Val Glu Gly Asp Ile Leu Ser Gly Val Cys Phe Val Gly Gln Leu Asp Thr His Ser  421

CTG GGC GCG TTC CTG ATC CTT CCA CTC TGC ATT TAT CTC TCG ATC GGA GCA CTA TTC CTG 1920
Leu Gly Ala Phe Leu Ile Leu Pro Leu Cys Ile Tyr Leu Ser Ile Gly Ala Leu Phe Leu  441 M5

CTG GCC GGA TTT ATT TCG CTT TTC CGG ATC CGG ACA GTT ATG AAA ACG GAC GGA AAG AGG 1980
Leu Ala Gly Phe Ile Ser Leu Phe Arg Ile Arg Thr Val Met Lys Thr Asp Gly Lys Arg  461

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Thr Asp Lys Leu Glu Arg Leu Met Leu Arg Ile Gly Phe Phe Ser Gly Leu Phe Ile Leu  481 M6

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CCG GGA TCT CCA GAA GCC CGC CCC ATC TTT CAG ATC TTT ATG GTC AAG TAC CTT TGC TCC 2220
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CGG AAC TTC GTG GAG AGG TTG CAG GGC AAG GAG CCC CGG ACC CGG GCG CAG GCG TAC GTC 2340
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ter
GCG GCT AAC GAA AGT TAC TTA GAG TTT AGC ACA TAG ACG TTC CCT ATG CCC AGA AAA AAA 2460
AAA AAA
```

FIG. 1 The sequence of the insert of the cDNA clone CVC22. The 2,466-bp sequence CVC22 is shown with the deduced translation product from the single long open reading frame. This cDNA clone was isolated from a library constructed by L. Kauvar[18] from poly(A)$^+$ RNA isolated from early pupae. The open reading frame has typical *Drosophila* codon usage, and the CAAA sequence preceding the ATG translation start sequence fits the *Drosophila* consensus sequence of C/AAAA/C[19]. There are termination codons in all three reading frames upstream from this start site. There is no typical poly(A) addition sequence (AATAAA) upstream of the 3′ oligo(A) sequence, and the 3′ untranslated region is relatively short, ~120 nucleotides. A more extensive cDNA clone analysis W. J. Park and P.N.A., unpublished results) has shown that CVC22 is missing over 1.3 kb of sequence of its 3′ end. The analysis of additional cDNA clones from other libraries has not revealed any additional *fz* mRNA species in larvae or pupae. The putative transmembrane domains are underlined and labelled. Sites of potential *N*-linked glycosylation are marked by an asterisk. The cDNA sequences were subcloned into the Bluescript vector (Stratagene), a set of processive deletions made, and single-stranded templates sequenced using the Sequenase reagents (US Biochemicals). The entire sequence was determined in both directions.

membrane domains *in vivo*, then the Fz protein would contain four extracellular and four intracellular domains. The largest of these would be the extracellular N-terminal domain. The sequences of three transmembrane domains (M4, M5 and M6, Fig. 1) are interesting because they contain a proline. Prolines are found in many transmembrane domains of membrane transport proteins[10] and cell-surface receptors[11], whereas they are excluded from the transmembrane domains of other classes of membrane proteins, such as viral envelope proteins[10]. There is no striking sequence similarity between Fz and any of the sequences in the NBRF and translated GENBANK data bases[12].

The function of the *fz* locus is required for the transmission of polarity information. The predicted structure of the Fz protein suggests is could do this either by transporting a polari morphogen[13] across cell membranes, or by mediating dire cell-cell interactions[14,15]. The *fz* locus function is also require for cells to be able to respond to polarity information. Th presence of seven putative transmembrane domains in the F protein is notable as seven transmembrane domains are foun in the large family of G-protein-coupled membrane recep tors[11,16,17]. Although Fz does not have substantial sequenc similarity to the products of any members of this gene family it does seem to have topological similarity. It is therefore possibl that Fz functions by a G protein to transduce polarit information to the cytoskeleton during hair and bristl development.

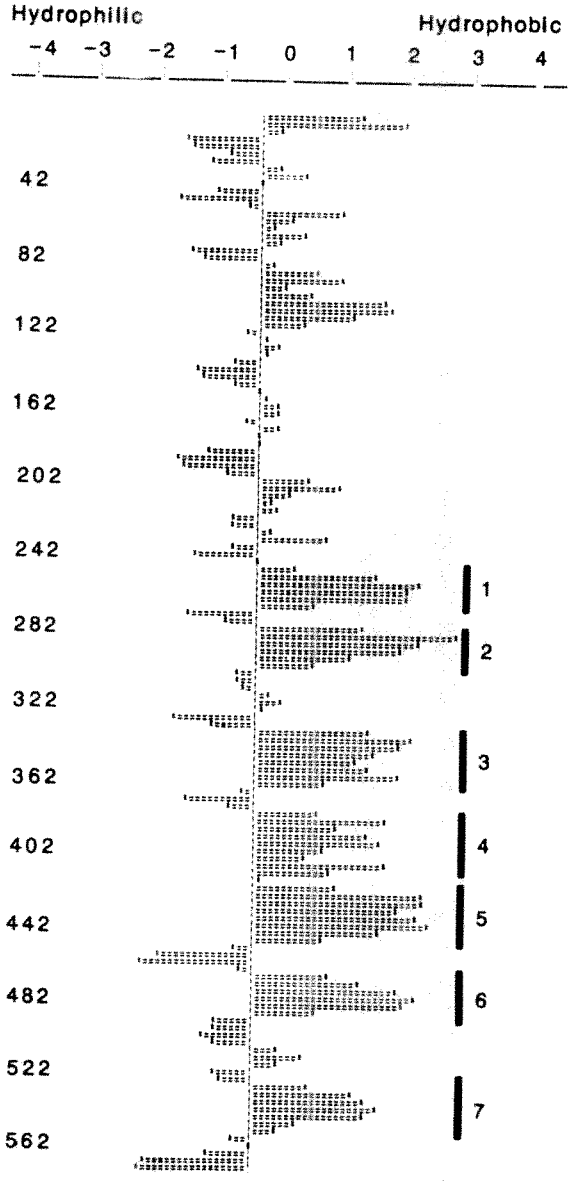


FIG. 2 Hydropathy plot of the Fz protein. Hydropathy of the deduced protein sequence was analysed as described in ref. 7, using windows of 9 and 19 residues, and by the GES approach[8] with a window of 20 amino acids. The plot shown was generated by the approach of Kyte and Doolittle[7] using a window of 9. The data is compressed four times, thus each point is an average for four residues. All analyses gave reasonably similar results except that the relatively hydrophobic region centred around amino acid 110 shown here came out much less hydrophobic when the GES approach was used (and somewhat less hydrophobic when the Kyte and Doolittle approach was used with a window of 19), so this region probably does not represent a transmembrane domain. The location of the seven proposed transmembrane domains is shown (1 to 7).

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A *Pseudomonas* thrives in high concentrations of toluene

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TOLUENE, like many organic sovents, is highly biotoxic and kills most microorganisms at low concentrations (0.1% v/v). It is often used therefore to sterilize microbial cultures and lyse bacterial cells in the assay of bacterial enzymes[1–3]. The physiological basis of such solvent toxicity, however, remains poorly characterized. Although some microorganisms, including *Pseudomonas*[4–6], *Achromobacter*[4] and *Nocardia*[7], can assimilate toluene, their tolerance for the solvent is less than 0.3% (v/v). We report here the discovery of a variant strain of *Pseudomonas putida* which is capable of growing in media culture containing more than 50% (v/v) toluene or high concentrations of cyclohexane, xylene, styrene and heptanol. By studying this unusually tolerant strain we show that the relative toxicities of different solvents are determined by their polarities.

We isolated the toluene-resistant bacterium from Japanese soil by selecting for microbial growth in a nutrient medium containing toluene. To isolate toluene-resistant microorganisms, a small amount of soil was suspended in sterile water and 0.2 ml of this suspension transferred to test tubes containing 5 ml of a nutrient medium comprising 0.1% glucose, 0.25% yeast extract (Difco Lab.) and 0.5% polypeptone (Difco Lab.), at pH 7.0. Toluene was then added to a final concentration of 30% (v/v), the test tubes were plugged with butyl-rubber, and the cultures incubated at 37°C for one week in a test-tube shaker. From the

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750 soil samples collected, one strain, IH-2000 (FERM BP-1751) which grew well in the toluene medium, was isolated from soil in Kyushu, southern Japan. Subsequent characterization of this bacterium showed that it is aerobic, motile, gram-negative and rod-shaped (0.8–1.0 by 2.0–4.0 μm), and has four polar flagella. The G and C content of its DNA is 62.5% and it reacts positively in catalase and oxidase tests, as well as being OF test-oxidative. From these and other morphological and biochemical characteristics of the strain, determined in accordance with standard methods[8], the isolate IH-2000 was identified as a strain of *P. putida*, which differs from other strains only by its resistance to toluene. Unlike *P. putida* strains which carry the TOL plasmid, strain IH-2000 is however, unable to utilize toluene as a nutrient.

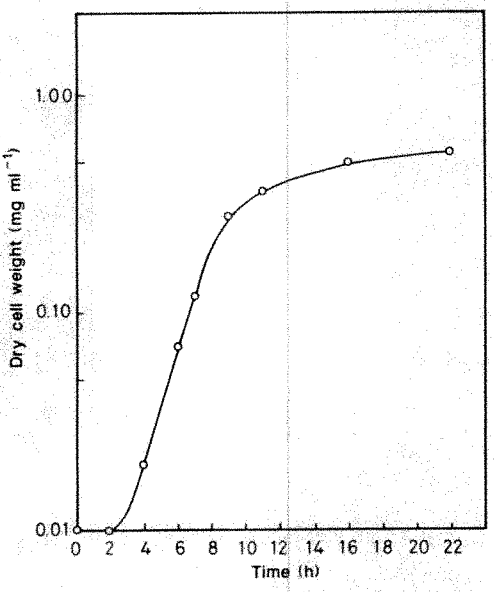


FIG. 1 Growth curve of strain IH-2000 in the LB medium containing 30% toluene.

By contrast with other strains including *P. putida* IFO 3738 and *P. putida* IAM 1506, which when tested for solvent tolerance failed to grow in a culture medium containing 30% toluene, strain IH-2000 grew in emulsions containing more than 50% toluene and was actively motile in these culture media. The doubling time for growth in a Luria–Bertani (LB) medium was 1.22 h and 0.77 h in the presence of 30% toluene and in the absence of the solvent, respectively (Fig. 1). After 24 h, the LB–toluene medium contained 2×10^9 cells per ml. Furthermore, single cells of this strain formed colonies on a solid nutrient medium, overlaid with toluene, which remained viable for several weeks. This indicates that solvent tolerance is a stable phenotypic property of strain IH-2000.

To investigate the solvent tolerance genetically, we isolated solvent-sensitive mutants using the replica-plating method in conjunction with penicillin selection after treatment with 1-methyl-3-nitro-1-nitrosoguanidine (NTG). First, we made Leu and Trp auxotrophs from the IH-2000 strain by two NTG treatments. We then isolated three toluene-sensitive mutants from 3,000 colonies of mutant cells. Remarkably, one of these (IH-2124T) was sensitive to toluene but resistant to xylene and cyclohexane. We subsequently isolated one xylene-sensitive mutant (IH-2124TX) from 2,000 colonies of mutant cells derived from the toluene-sensitive strain. This strain was consequently sensitive to both toluene and xylene, but resistant to cyclohexane. From the IH-2124TX strain, a cyclohexane-sensitive mutant (IH-2124TXC) which remained hexane-resistant was isolated. Each of these mutants retained the Leu and Trp auxotrophic markers. The toluene-sensitive mutant, for which the frequency of back mutation was less than 10^{-9}, was very stable. The xylene-sensitive and cyclohexane-sensitive mutants exhibited frequencies of back mutation of 10^{-7} to 10^{-8} respectively. In addition to toluene, these strains tolerated other toxic solvents including aliphatic hydrocarbons, alicyclic hydrocar-

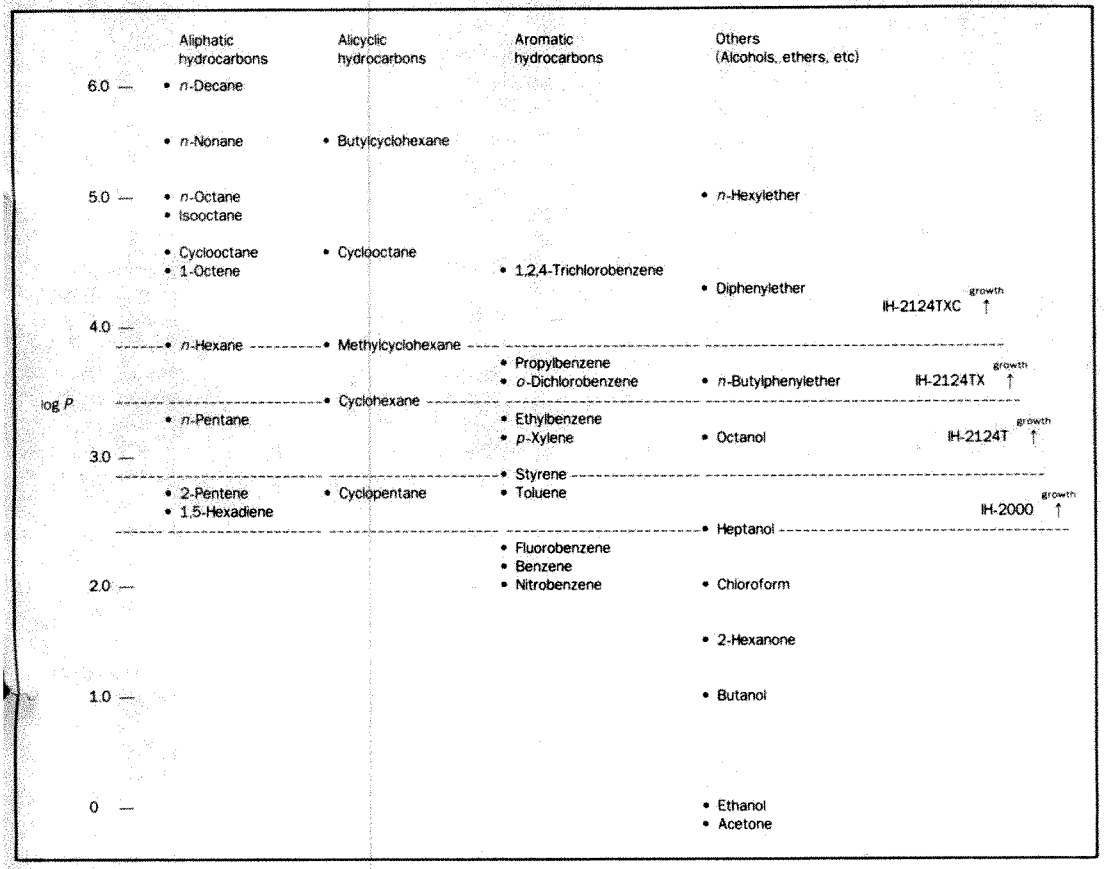


FIG. 2 Correlation between log *P* values and growth limits of the strain IH-2000 and its mutants in media containing solvents. Log *P* values were calculated using the CLOG program, 3.33 (Pomona College, USA).

TABLE 1 Solvent tolerance of bacterial strains and mutants

	log *P*	IH-2000	IH-2124T	IH-2124TX	IH-2124TXC	*E. coli* IFO 3806	*P. putida* IFO 3738	*P. fluorescens* IFO 3507	*Achromobacter delicatulus* IAM 1433	*Agrobacterium tumefaciens* IFO 3058	*Alcaligenes faecalis* JCM 1474	*Aeromonas hydrophila* JCM 1027	*B. subtilis* AHU 1219	*Corynebacterium glutamicum* JCM 1318	*S. uvarum* ATCC 26602	No. tolerant strains
Dodecane	7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Decane	6.0	+	+	+	+	+	+	+	+	+	+	+	+	−	−	12
Nonane	5.5	+	+	+	+	+	+	+	+	+	+	+	+	−	−	12
n-Hexylether	5.1	+	+	+	+	+	+	+	+	+	+	+	+	−	−	12
Octane	4.9	+	+	+	+	+	+	+	+	+	+	+	+	−	−	12
Isooctane	4.8	+	+	+	+	+	+	+	+	+	+	+	−	−	−	11
Cyclooctane	4.5	+	+	+	+	+	+	+	+	−	+	+	−	−	−	10
Diphenylether	4.2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	8
n-Hexane	3.9	+	+	+	+	+	+	+	+	−	−	−	−	−	−	8
Propylbenzene	3.8	+	+	+	−	+	+	+	−	−	−	−	−	−	−	6
o-Dichlorobenzene	3.6	+	+	+	−	−	+	+	−	−	−	−	−	−	−	5
Cyclohexane	3.4	+	+	+	−	−	+	+	−	−	−	−	−	−	−	5
Ethylbenzene	3.3	+	+	−	−	−	+	−	−	−	−	−	−	−	−	3
p-Xylene	3.1	+	+	−	−	−	+	−	−	−	−	−	−	−	−	3
Styrene	2.9	+	+	−	−	−	−	−	−	−	−	−	−	−	−	2
Toluene	2.8	+	−	−	−	−	−	−	−	−	−	−	−	−	−	1
Heptanol	2.4	+	−	−	−	−	−	−	−	−	−	−	−	−	−	1
Benzene	2.1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0
Limiting log *P* value for growth		2.4	2.9	3.4	3.9	3.8	3.1	3.4	3.9	4.8	4.5	4.5	4.9	7.0	7.0	

+, growth −, no growth

bons, aromatic hydrocarbons, alcohols and ethers. The solvent tolerance properties of the IH-2000 and its mutants are summarized in Table 1.

Figure 2 shows the correlation between the growth of strain IH-2000 and the polarity of the solvent. The parameter log *P*, where *P* is the partition coefficient of a given solvent in an equimolar mixture of octanol and water, is used as a quantitative index of solvent polarity. It is evident that although the strain grows in solvents whose log *P* values are greater than or equal to 2.4, such as heptanol (log $P = 2.4$), toluene (log $P = 2.8$) and n-octane (log $P = 4.9$), it does not grow in fluorobenzene, benzene and butanol whose log *P* values are 2.3, 2.1 and 0.8, respectively. It seems therefore that a critical point is reached beween log *P* values of 2.4 and 2.3, at which the solvent is polar enough to prevent growth. The concept of such a tolerance limit can be extended to predict the growth of other microbial species in solvent-saturated environments (see Table 1).

These observations indicate that the nature of the interaction between the solvent and the cell surface that limits growth is at least partly determined by solvent polarity. Moreover, the unusually high tolerance of strain IH-2000 for toluene and other solvents probably reflects the presence of cell surface components that are unique to the strain. With a view to characterizing these components and their genetic basis, we have already isolated, from the chromosomal DNA of strain IH-2000, a 10-kilobase fragment (data not shown) which contains genes for solvent tolerance. □

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Phase variation in *Bordetella pertussis* by frameshift mutation in a gene for a novel two-component system

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***BORDETELLA pertussis*, the aetiological agent of whooping cough, coordinately regulates the expression of many virulence-associated determinants, including filamentous haemagglutinin, pertussis toxin, adenylyl cyclase toxin, dermonecrotic toxin and haemolysin. The coordinate regulation is apparent in the repression of synthesis of these determinants in response to environmental stimuli[1,2]; a phenomenon known as antigenic or phenotypic modulation. *B. pertussis* also varies between metastable genetic states, or phases. There is a virulent phase in which virulence-associated determinants are synthesized, and an avirulent phase in which they are not[3]. Previous studies[4,5] have shown that a genetic locus, *vir*, is required for expression from many virulence-associated loci, and that replacing the cloned *vir* locus in *trans* can restore the virulent phase phenotype to spontaneously occurring avirulent phase strains. Here, we show that phase variation in one series of strains is due to a frameshift mutation within an open reading frame that is predicted to code for a Vir protein product. The deduced protein sequence is similar to both components of the 'two-component' regulatory systems which control gene expression in response to environmental stimuli in a range of bacterial species.**

Tohama I is a virulent phase *B. pertussis* strain isolated from a pertussis patient in Japan[6]. The ancestry of all *B. pertussis* strains used in this study can be traced to this strain, and their

elationship is shown in Fig. 1. We have shown previously that 14.7-kilobase (kb) *Bam*HI fragment from BP338 containing he *vir* locus can restore the virulent phase phenotype to BP369-3 vhen returned in *trans*[5]. We repeated this experiment for the ɔther strains shown in Fig. 1 by cloning the homologous *Bam*HI ragment from BP326, BP368-3, BP369-3, BP370-1 and BP371-1 nto the broad host-range cloning vector pRK290 (ref. 7) and ransferring the resultant plasmids by conjugation to the ıvirulent phase strains BP326, BP369-3 and BP371-1. When the ɔir locus transferred had been cloned from one of the virulent ɔhase strains BP338, BP368-3 or BP370-1, the resulting excon- ugant colonies had a typically Vir^+ phenotype, being compact hemispherical (domed), and with a clear zone of haemolysis on Bordet–Gengou agar. When the *vir* locus was from BP326, BP369-3 or BP371-1, a Vir^- phenotype was obtained (flat and nonhaemolytic).

We examined the virulent phase (*vir*$^+$ form) and the avirulent phase (*vir*$^-$ form) of the cloned *vir* locus to see if there was a physical difference, such as an inversion or deletion that could explain the genetic differences. None were apparent after restriction enzyme analysis with *Eco*RI, *Sal*I or *Pst*I, or electron microscopic heteroduplex analysis (data not shown). We therefore designed an experiment to map the location of this difference by genetic means.

We constructed a set of deletions of the *vir* locus of BP338 *in vitro* (Fig. 2), and tested them for their ability to restore the *Vir*$^+$ phenotype in two different ways. For the test in *trans*, each derivative was cloned into pRK290 and returned by conjugation to a virulent phase *B. pertussis* strains. None of the four deletions tested contained an intact *vir* locus, as they were all negative in *trans* (data not shown). To test for restoration of the *Vir*$^+$ phenotype by recombination, the same deletion fragments were cloned into pSS853, a vector that confers kanamycin resistance. This vector can be transferred by conjugation into *B. pertussis* but cannot replicate there autonomously. Thus, exconjugants can be selected in which the plasmid has been integrated into the *B. pertussis* chromosome by selection for kanamycin resistance. This integration will probably occur by homologous recombination within an area of homology; in the case of the *vir* deletion derivatives, within the cloned *vir* DNA. If the deletion derivative being tested contains the site of the difference between the *vir*$^+$ and *vir*$^-$ forms then some of the exconjugants arising from a mating will be Vir^+ due to crossover between this site and the end-point of the deletion. If the deletion derivative does not contain the site of this difference, only Vir^- colonies will be obtained. As shown in Fig. 2, two of the deletions could restore the virulent phase phenotype by recombination. These data indicate that the difference between the *vir*$^+$ and *vir*$^-$ forms of the *vir* locus lies within the deletion interval defined by deletions 856 and 858, within the *Sal*I fragment D. The same results were obtained regardless of which avirulent phase strain was the recipient; hence, the *vir*$^-$ lesion is within this deletion

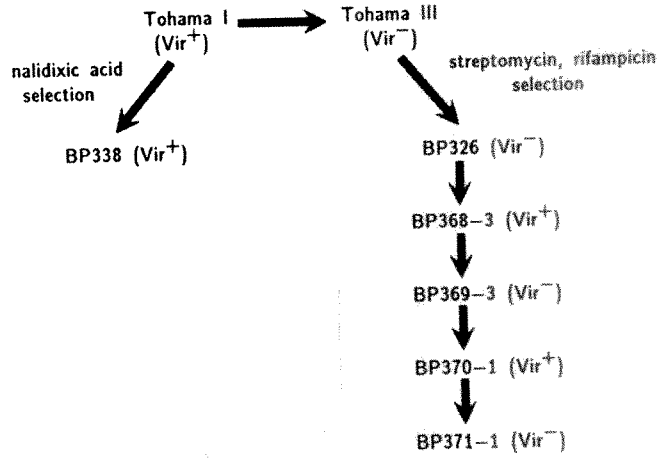


FIG. 1 Pedigree of *B. pertussis* strains used in this study. Tohama I was isolated from a patient in Japan[6]. BP338 is a spontaneous nalidixic acid-resistant derivative of Tohama I[1,2]. Tohama III was isolated as a spontaneous variant of Tohama I after passage *in vitro* (Y. Sato, personal communication). BP326 is a spontaneous rifampicin and streptomycin-resistant derivative of Tohama III[1,2]. BP368-3 and BP370-1 were isolated as spontaneously occurring haemolytic variants of BP326 and BP369-3, respectively[4,8]. BP369-3 and BP371-1 were isolated from BP368-3 and BP370-1, respectively, by erythromycin selection for avirulent phase derivatives[4,5].

interval in BP326, BP369-3 and BP371-1. To establish further that the difference between the *vir*$^+$ and *vir*$^-$ forms was contained within *Sal*I restriction fragment D, this fragment was used in a double crossover recombination experiment to rescue the Vir^- phenotype. It was cloned into pRTP1, a vector designed for the replacement of chromosomal sequences with cloned and/or modified ones[8] (Fig. 2). The resulting plasmid was modified further by the addition of a gene encoding kanamycin resistance to improve the selection for exconjugants. This plasmid, pSS1064, was transferred by conjugation to BP326, BP369-3 and BP371-1. The resulting kanamycin-resistant *B. pertussis* derivatives were of the avirulent phase, as judged by colony morphology and we assumed that the plasmid had integrated via a single crossover within the cloned *Sal*I fragment. In a sampling of these *vir*$^-$ exconjugants, we selected for a second crossover event by selecting for loss of the integrated plasmid by virtue of the streptomycin sensitivity phenotype it conferred. The colonies arising after streaking on streptomycin-containing media were both Vir^+ and Vir^-, as expected. The two types of colonies were obtained in approximately equal numbers. The frequency at which *vir*$^+$ derivatives arise by this method is at least 1,000-fold higher than the frequency at which they arise spontaneously. This experiment confirmed that the site of the functional difference in the two forms of the *vir* locus is within the *Sal*I fragment D.

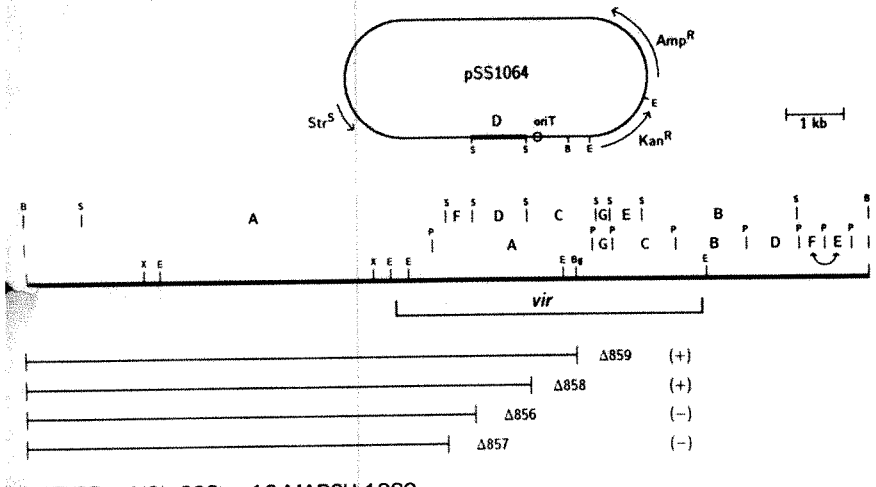


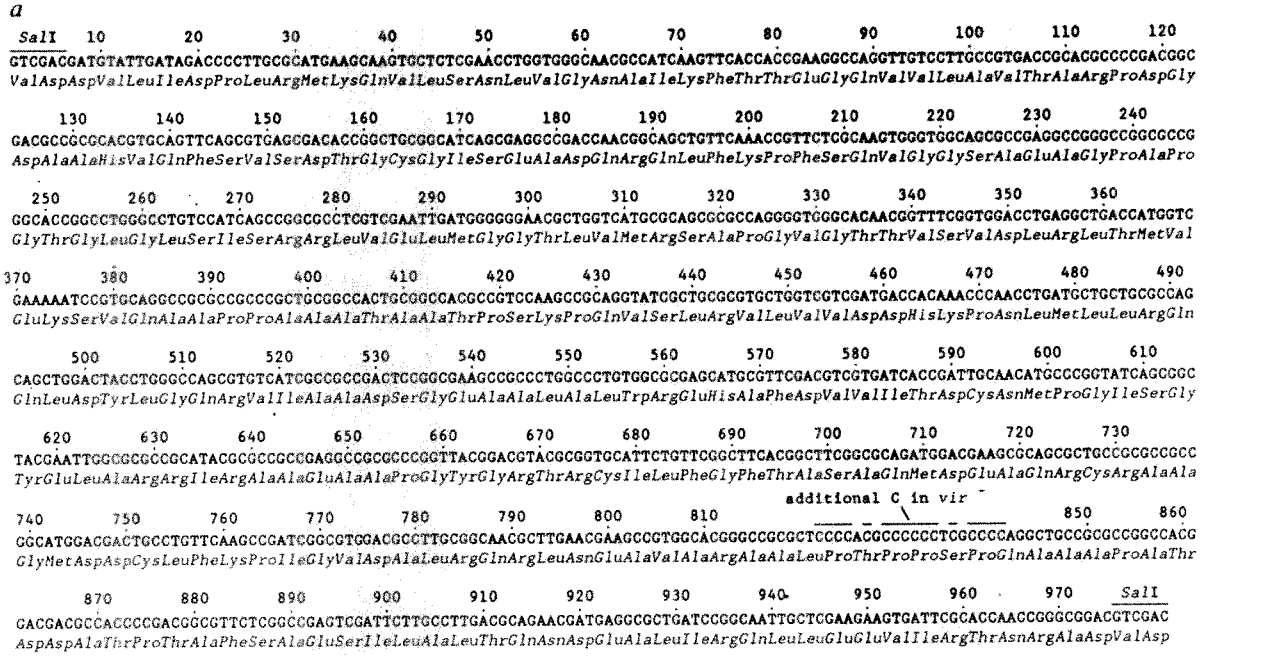
FIG. 2 Restriction map of the *vir* locus of *B. pertussis*. Restriction enzyme abbreviations: B, *Bam*HI; E, *Eco*RI; P, *Pst*I; S, *Sal*I; X, *Xho*I. The bracket below the map shows the extent of the *vir* locus determined by insertion and deletion mapping in *B. pertussis* (manuscript in preparation). DNA sequences remaining in the deletion derivatives are shown below the map: '+' indicates that a given deletion was capable of restoring, by recombination, the virulent phase phenotype (domed and haemolytic) when cloned into pSS853 and transferred from *Escherichia coli* strain SM10 (ref. 13) to BP326 by conjugation. pSS853 is a derivative of pBR322 containing *oriT* from RK2, and a kanamycin-resistance gene. Shown above the map is the structure of pSS1064; derived by cloning the *Sal*I fragment D of *vir* from BP338 into the vector pRTP1 (ref. 8) and subsequent addition of a kanamycin-resistance cassette.

To define the precise difference between the *vir*$^+$ and the *vir*$^-$ allelic forms, the DNA sequence of the *Sal*I fragment D was determined (Fig. 3*a*). The only difference observed between the two *vir*$^+$ forms of BP338 and BP368-3 and the *vir*$^-$ form of BP326 was the presence of an extra C residue in a run of six C residues. The sequence of this C-rich region from BP369-3, BP370-1 and BP371-1 was also determined. In these strains as well, the *vir*$^+$ form contained six C's and the *vir*$^-$ forms contained seven C's. The difference between the two forms of the *vir* locus is therefore a frameshift mutation. This mutation occurs in a 'classical' context for frameshift mutations: runs of a particular nucleotide, especially G or C (ref. 9).

Figure 3*b* shows that, if the open reading frame is read from right to left with reference to Fig. 2, there is significant sequence similarity between the beginning of the predicted Vir protein sequence and the C-terminal sequences of members of a class of bacterial regulatory proteins that constitute one component of the 'two-component' systems which regulate gene expression in response to environmental stimuli in a wide variety of bacteria[10]. This 'sensor' component is often localized to the bacterial inner membrane and is involved in sensing the environment. The other 'regulator' component is thought to interact with this 'sensor' and to exert its effects in response to signals received from it. Recently, this interaction has been shown to involve phosphorylation of the regulator component by the sensor component in some of these systems[11]. Interestingly, the second part of the Vir sequence has significant similarity with that of the 'regulator' component. These data suggest that one of the genes within the *vir* locus encodes a protein that can respond to environmental signals with changes in the expression of virulence-associated factors. What is novel about this putative *vir* gene product is that both components of a 'two-component' system are apparently incorporated into a single protein. This combination of sensor and regulator modules within a single protein has recently been recognized in other bacterial signalling proteins[25].

If the difference between the *vir*$^+$ and *vir*$^-$ forms is indeed a frameshift mutation, a frameshift mutagen would be expected to increase the rate of conversion between these two forms. To test this hypothesis, BP326 was grown for several generations in Stainer–Scholte broth containing varying amounts of the frameshift mutagen ICR-191. The proportion of Vir$^+$ colonies obtained when plated onto solid media was influenced in a dose-dependent way by the concentration of ICR-191 (Fig. 4).

a



b

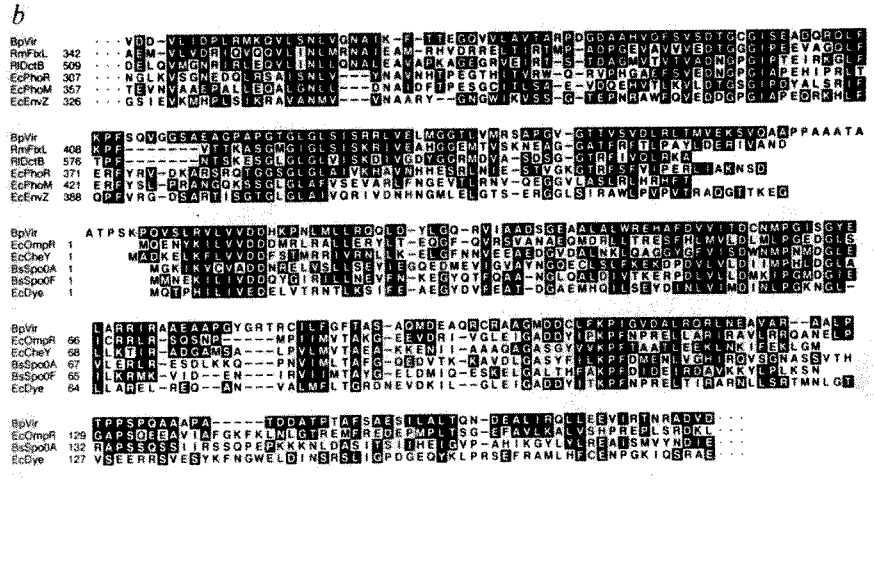


FIG. 3 *a*, DNA sequence of the *Sal*I restriction fragment D from BP338 and BP368-3. The sequence is read from right to left with respect to Fig. 2, and was determined by the dideoxy chain-termination method using M13 phage templates and synthetic primers to the *vir* sequence. The sequence of both strands was determined. The C-rich region is overlined. *b*, Sequence comparison of the predicted portion of a *vir* product and some examples of members of 'two-component' regulatory systems. 'Sensor' sequences are FixL from *Rhizobium meliloti*[14], DctB from *Rhizobium leguminosarum*[15], PhoR from *E. coli*[16], PhoM from *E. coli*[17], and EnvZ from *E. coli*[18]. 'Regulator' sequences are OmpR from *E. coli*[18], CheY from *E. coli*[19], Spo0A from *Bacillus subtilis*[20], Spo0F from *B. Subtilis*[21] and Dye from *E. coli*[22]. Alignments were made using the FASTP algorithm[23]. Boxed residues indicate a similar amino-acid residue between Vir and the other sequences. Similar residues are those belonging to the groups: PAGST; HKR; QNED; ILVMFYW (single-letter code). Numbers indicate amino-acid residue number within a protein. Dots indicate that the protein sequence extends beyond that shown.

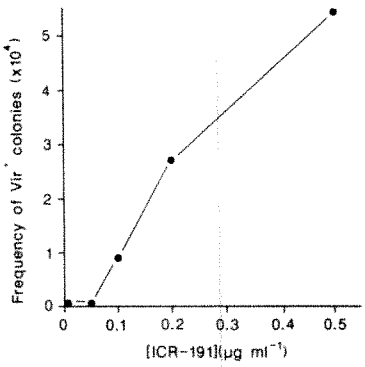


FIG. 4 Reversion of the avirulent phase phenotype of BP326 by treatment with the frameshift mutagen ICR-191. The procedure was essentially as previously described[24]. A culture of BP326 grown in Stainer–Scholte broth was diluted by 10^{-4}. Aliquots (0.1 ml) were added to 3 ml of the same medium containing different concentrations of ICR-191 and incubated in the dark at 35 °C, with shaking, for 5–7 days. Cultures showing visible turbidity were diluted and plated on Bordet–Gengou agar to obtain 10^3–10^4 colonies per plate. After growth for 5 days, plates were examined for haemolytic colonies.

Similar results were obtained for BP369-3 and BP371-1. These data support the hypothesis that phase variation in this series of strains occurs via a frameshift mutation in a *vir* gene.

An important question arising from our results is whether this frameshift mutation represents a true 'programmed' genetic event reflecting an evolved site-specific mechanism. The exact nucleotide sequence requirements at the site of the mutation are not yet known. It is worth noting that in *B. pertussis*, an organism with a relatively high (G+C) content, a run of six G or six C residues is, as expected, not uncommon. The sequence around the site of the frameshift mutation is striking however, in its abundance of GC pairs and in its near symmetry. It will be interesting to test whether the groups of C residues outside the core six C residues affect the frequency of phase variation. This frequency varies with the strain used and is relatively high in the strains which have descended from Tohama III (about 10^{-3} for the vir^+ to vir^- transition[4]). In contrast, the frequency at which BP338 undergoes this transition is about 10^{-6}. In an attempt to determine the basis for this difference, we are examining the reversion of other frameshift mutations in these two backgrounds to determine whether this is a general effect on frameshift mutation/reversion. The relevance of our results, derived from work on laboratory strains, to bacteria in their natural environment is an important issue. It is unclear what the role of phase variation is in the natural biology of this highly adapted pathogen. This phenomenon has been observed after passage of *B. pertussis* isolates *in vitro* in most cases. The isolation of Vir^-, or phase III, organisms from patients late in infection has been reported however[6]. We are currently examining vir^+/vir^- pairs derived from other isolates to determine if the frameshifting we have described represents a common mechanism for phase variation in *Bordetella* species. □

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A new strategy for the generation of catalytic antibodies

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THE high binding affinity and specificity of antibodies for a wide range of ligands has recently been exploited in the generation of catalysts for acyl-transfer reactions[1–7], carbon–carbon bond forming[8,9] and carbon–carbon bond cleaving reactions[10]. In addition, a number of strategies are emerging for the generation of catalytic antibodies including transition state stabilization[1–6], catalysis by approximation[7,8], and the introduction of catalytic groups or cofactors into antibody combining sites[10–13]. An important goal in the design of catalytic antibodies is the development of general rules relating hapten structure to the corresponding catalytic groups in the antibody combining site. We report here that electrostatic interactions between a hapten and the complementary antibody[14,15] can be exploited to generate catalytic amino-acid side chains in an antibody-combining site. The antibody-catalysed reaction, a β-elimination reaction, exhibits saturation kinetics, substrate specificity, competitive inhibition by hapten, and specific inactivation by a reagent that modifies carboxylate residues.

To expand the scope of antibody catalysis we targeted reactions involving proton abstraction from a carbon centre. This class of reactions includes elimination and isomerization reactions and aldol and Claisen condensations[16–18]. Proton abstraction in enzymes is often performed by carboxylate side chains of glutamate or aspartate amino acids. These residues typically display higher than normal pKa values (6.5–8.2) in the hydrophobic active sites of enzymes[19]. Aspartate and glutamate residues have been generated in the combining sites of antibodies by taking advantage of charge complementarity between haptens and the corresponding antibodies[14,15]. Consequently, we reasoned that an antibody-combining site could be generated that contains a carboxylate group within bonding distance of an abstractable substrate proton by using the appropriate positively charged hapten. Importantly, the position of a charged group such as alkyl ammonium ion in the hapten should reflect that of the target C–H group in the substrate.

To test these notions, monoclonal antibodies generated to hapten H1 (Fig. 1) were assayed for their ability to catalyse H–F elimination from the fluorinated substrate S2 (Fig. 1). The hapten contains a positively charged ammonium ion replacing the α-CH_2 group of the substrate (the tertiary nitrogen obviates competing lactam formation during the coupling of H1 to pro-

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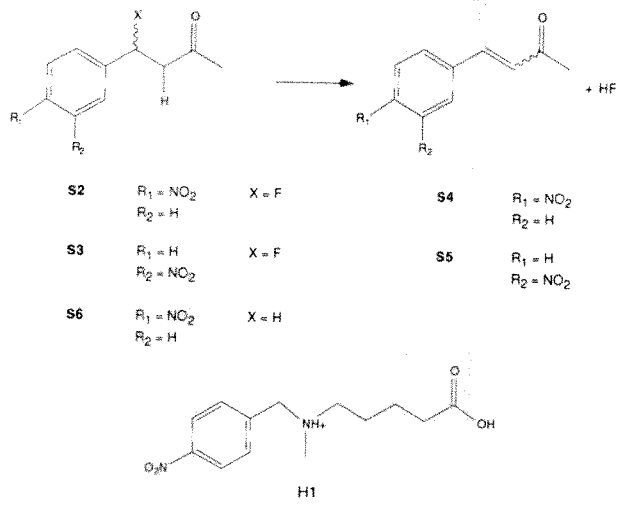


FIG. 1 Monoclonal antibodies elicited against the (KLH) conjugate of H catalyse the conversion of S2 or S4. Synthesis of H1 was accomplished b treatment of *p*-nitrobenzaldehyde and ∂-aminovaleric acid with 0. equivalents (eq.) of sodium cyanoborohydride ($NaCNBH_3$) in methanol at 25 ° to afford N(*p*-nitrobenzyl)-∂-aminovaleric acid which was purified by recrysta lization from H_2O/acetone. This compound was then treated with 37 aqueous formaldehyde and 2 eq. $NaCNBH_3$ in H_2O at 25 °C to afford H1 whic was purified by silica-gel chromatography. Compound H1 was coupled throug amide linkages to KLH by treatment with the *N*-hydroxysuccinimide ester c H1 to afford the KLH conjugate with an epitope density of 18 per carrie monomer as determined by the method of Habeeb[24]; protein concentratio was determined by the method of Lowry[25]. Synthesis of S2 was carried ou by treatment of *p*-nitrobenzaldehyde with a catalytic amount of 1% aqueou NaOH in acetone at 0 °C to give the intermediate (*R, S*) 4-hydroxy-4-*p* nitrophenylbutan-2-one which after purification by silica-gel chromatography treatment with diethylaminosulphur trifluoride in CH_2Cl_2 at −78 °C an aqueous extraction afforded racemic S2. Treatment of the intermediat alcohol with 1.1 eq of H_2SO_4 (1N) in acetone at 25 °C and purification b silica gel chromatography afforded the *trans* isomer of S4. Compounds S3 and S5 were synthesized analogously to S2 and S4 starting with 3-nitroben zaldehyde. Substrate analogue S6 was obtained by nitration of benzylacetone with $Cu(NO_3)_2 \cdot 3H_2O$ in neat trifluoroacetic anhydride at 39 °C and purified by silica gel chromatography. All compounds were characterized by NMR, mass spectrometry, infrared and ultraviolet spectroscopy and elemental analysis

tein carriers for immunization). The fact that the hapten and substrate share a common recognition element, the *p*-nitrophenyl group, ensured that the antibody-binding affinity for the substrate would be reasonable. Moreover, replacement of hapten by substrate in the antibody-combining site should lead to an increase in the basicity of the catalytic carboxylate since a stabilizing salt bridge interaction is lost.

Antibodies were generated against hapten H1, which at physiological pH exists as a positively charged alkyl ammonium ion. The hapten was conjugated to the carrier proteins bovine serum albumin (BSA) and keyhole limpet haemocyanin (KLH) using the activated *N*-hydroxysuccinimide ester of H1 (ref. 20). Six monoclonal antibodies specific for H1 were produced *in vivo* and isolated by affinity chromatography on protein A-coupled Sepharose 4B as decribed previously[3]. Antibody purity was determined by 10–15% SDS polyacrylamide gel electrophoresis with Coomasie blue staining[21]. Of these six IgGs isolated, four accelerated the β-elimination reaction of S2 to S4 (Fig. 1) and were completely inhibitable by the addition of free

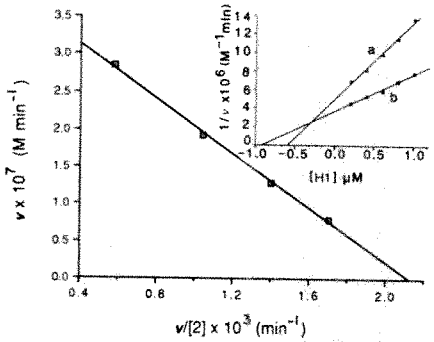


FIG. 2 Eadie–Hofstee plot for the β-elimination conversion of S2 to S4, catalysed by 43D4-3D3. Velocities were determined spectrophotometrically by measuring the initial absorbance increase at 330 nm: S2, $\lambda_{max} = 282$ nm (3.99); S4 $\lambda_{max} = 312$ nm (4.43); ΔE(S4–S2) (330 nm) = 16,820 $cm^{-1} M^{-1}$. The concentration of 43D4-3D3 was 2.00 μM as determined by absorbance at 280 nm using E(1 cm, 0.1%) = 1.37 and M_r = 150,000 for IgG. 43D4-3D3 was preincubated at 37 °C in 10 mM bis [2-hydroxyethyl]imino-tris-[hydroxymethyl]-methane (bis-Tris), 100 mM NaCl, pH 6.0. The reaction was initiated by adding 10 μl of a stock solution of S2 in CH_3CN to give a final CH_3CN concentration of 2%. The uncatalysed rate was measured under the same conditions, affording a $k_{uncat} = 1.30 \times 10^{-4}$ min^{-1}. Inset: Dixon plot[26] of inhibition of 43D4-3D3 by H1. Antibody concentration, 2.01 μM. Data was obtained at two concentrations of S2: *a*, 208 μM; *b*, 514 μM. Buffer conditions as above.

hapten H1. Such hapten-inhibitable rate acceleration suggests that the catalysis is due to binding of the substrate in the antibody-combining site (non-specific antibodies did not catalyse the elimination reaction). The fact that 66% of these antibodies were catalytic demonstrates the generality of this strategy for producing catalytic antibodies. One of the four antibodies (43D4-3D3) was characterized further.

The reaction catalysed by 43D4-3D3 obeys classical Michaelis–Menten kinetics: a k_{cat} of 0.193 min^{-1} and a K_M of 182 μM for substrate S2 were measured at pH 6.0 (Fig. 2). Both the *cis* and *trans* isomers of enone S4 are formed in the antibody-catalysed conversion of racemic S2, as determined by high pressure liquid chromatography (HPLC) analysis. The catalysed reaction is also competitively inhibited by hapten H1 (K_i = 290 nM) (Fig. 2 inset) demonstrating that the catalytic activity is associated with binding in the antibody-combining site. An unreactive substrate analogue *p*-nitrobenzylacetone (S6) is also a competitive inhibitor of the catalysed reaction ($K_i = 280$ μM). As expected, the antibody-catalysed reaction is substrate-specific, in accordance with the characteristic specificity of antibodies for their ligands[15]. The K_M and k_{cat} values for 4-fluoro-4-*m*-nitrophenylbutan-2-one (S3) are 571 μM and 0.079 min^{-1}, respectively ($k_{cat}/K_M = 1.38 \times 10^2$ M^{-1} min^{-1}) at pH 6.50, compared with a k_{cat} of 0.304 min^{-1} and K_M of 214 μM ($k_{cat}/K_M = 1.42 \times 10^3$ M^{-1} min^{-1}) for S2 at this pH.

The identity of the catalytic amino-acid side chain in the antibody combining site was probed by two methods. The pH dependence of k_{cat} shows the classical profile for catalysis attributable to a single titratable group (Fig. 3). The pK_a for the active site residue was determined to be 6.2. This value is very close to the pK_a for the active-site Glu 135 in carboxypeptidase A ($pK_a = 6.5$), which is known to be responsible for the catalysis of a similar β-elimination reaction (not the physiologically relevant reaction for this enzyme)[22]. The maximum k_{cat} value obtainable by the antibody results when the residue responsible

TABLE 1 Effect of DAA treatment of 43D4-3D3 on catalysis of S2 to S4

Sample	v (M^{-1} min^{-1})*	% activity remaining
Untreated	3.41×10^{-7}	100
DAA treated†	7.97×10^{-8}	23.4
DAA treated† + 2 mM S6	2.79×10^{-7}	82.0

* 1.5 μM 43D4-3D3, 540 μM S2, pH 6.0, 10 mM Bis-Tris, 100 mM NaCl.
† Following the procedure described in ref. 14.

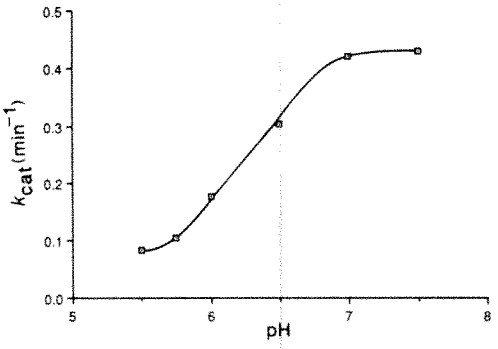


FIG. 3 The k_{cat} versus pH profile for conversion of S2 to S4 catalysed by 43D3-3D3. At each pH the k_{cat} value was obtained from a Lineweaver–Burke plot. The buffer used was 10 mM bis-Tris, in the presence of 100 mM NaCl.

for base catalysis is fully deprotonated. The value of k_{cat}(max), determined from the pH profile by plotting k_{cat} versus $k_{cat}[H^+]$, is 0.458 min^{-1} (ref. 23).

Chemical modification confirmed that an aspartate or glutamate residue is involved in the elimination reaction. Specific chemical modification of carboxylate residues was effected by treatment of 43D4-3D3 with diazoacetamide (DAA). DAA has been shown to react almost exclusively with carboxylate groups to form glycolamide ester linkages[14]. The antibody 43D4-3D3 retains only 23% of its catalytic activity after treatment with DAA. However, in the presence of competitive inhibitor S6 (2.0 mM), under otherwise identical conditions, 82% of its catalytic activity is retained. These results indicate that a carboxylate group could be located in or very near the binding site of 43D4-3D3.

The ability to generate antibody binding sites with specific catalytic groups or microenvironments provides a method for dissecting the contribution of various factors involved in enzymatic catalysis, such as transition-state stabilization, proximity effects, general acid-base, nucleophilic catalysis and ground state strain. Hapten H1 bears very little resemblance to the transition state for the conversion of S2 to S4; in fact it has the opposite charge of an E2 elimination transition state. This observation, together with the pH profile (Fig. 3), suggests that the observed catalysis is largely due to the presence of a catalytic base. The rate constant for acetate-catalysed conversion of S2 to S4, k_{OAc^-}, is 1.13×10^{-2} M^{-1} min^{-1} at 37 °C in 100 mM NaCl. The k_{cat}/K_M value of the antibody-catalysed reaction at pH 6 is 9.95×10^2 M^{-1} min^{-1}. The rate enhancement obtained by introduction of a carboxylate residue in a hydrophobic binding site compared with acetate free in solution is therefore 8.80×10^4 at the pH. This value is similar to that of 6×10^4 reported for an esterolytic antibody that ccontains an active site nucleophilic thiol[12]. The large rate enhancement due solely to base catalysis by a carboxyliate residue suggests that an antibody which combines transition state stabilization and base catalysis might attain rate accelerations similar to those of enzymes. Nuclear magnetic resonance experiments and resolution of the *R*, *S* stereoisomers of S2 are currently in progress to determine the stereochemistry of proton abstraction (pro*R*, pro*S*) and elimination (*syn*-coplanar, *anti*-coplanar).

This work represents the extension of antibody catalysis to a new class of reactions. Moreover, it should be possible to exploit hapten complementarity to generate antibodies which contain other catalytic groups and catalyse a variety of reactions of interest, including aldol and Claisen condensations, as well as amide and sugar hydrolyses.

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Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid-responsive element

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STEROID hormone receptors activate specific gene transcription by binding as hormone-receptor complexes to DNA enhancer elements termed hormone responsive elements (refs 1, 2 and references therein). A highly conserved 66-amino-acid region of the oestrogen and glucocorticoid receptors which corresponds to part of the receptor DNA-binding domain (region C) determines the specificity of target gene recognition[3–5]. This region contains two subregions (CI and CII), encoded in two separate exons (refs 1, 7 and references therein), that are analogous to the 'zinc fingers' of the transcription factor TFIIIA (reviewed in ref. 6). The N-terminal CI finger determines the recognition specificity of the hormone responsive element[8]. A chimaeric oestrogen receptor, in which the CI finger is replaced with the corresponding glucocorticoid receptor CI finger region, activates transcription from a reporter gene containing a glucocorticoid-responsive element, but not from a reporter gene containing an oestrogen-responsive element[8]. We report here that three amino acids located at the C-terminal side of the oestrogen receptor CI finger play a key part in this specificity.

In the reporter gene *vit-tk-CAT* (ref. 9), the 5'-flanking region of the *Xenopus* vitellogenin A2 gene (*vit*), which contains a consensus palindromic oestrogen-responsive element (ERE), is inserted upstream of the herpes simplex virus promoter for thymidine kinase (*tk*). In the mouse mammary tumour virus (MMTV) reporter gene MMTV-*CAT*, the glucocorticoid-

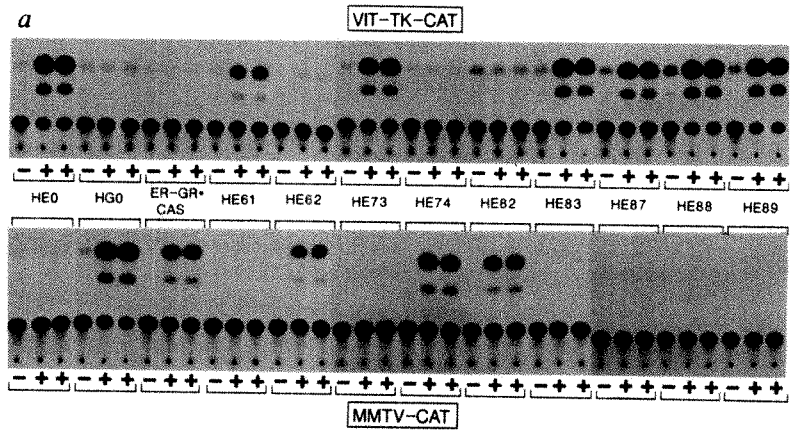
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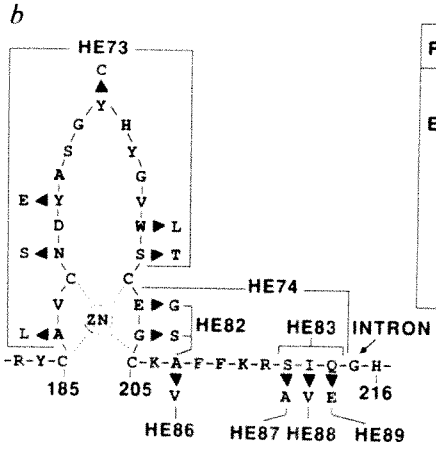
responsive element (GRE) in the long terminal repeat of MMTV is positioned upstream of the *CAT*-indicator gene. It can be seen from Figs 1 and 2 that co-transfection of HeLa cells with the wild-type human oestrogen receptor (hER) expression vector HEO resulted, as expected, in the activation of *vit-tk-CAT* expression (but not of MMTV-*CAT* expression) in the presence (+) of oestradiol; MMTV-*CAT* expression, however, was specifically stimulated by the human glucocorticoid receptor (hGR) expression vector HGO in the presence (+) of dexamethasone. Note that essentially the same variations in target gene specificities were observed for the various receptor mutants studied below when a reporter containing a consensus GRE (GRE-*tk-CAT*, see ref. 8) was used as a glucocorticoid receptor (GR) reporter instead of MMTV-*CAT* (data not shown).

In agreement with our earlier results, the chimaeric receptors ER-GR.CAS (refs 3, 4) and HE62 (ref. 8) (in which the 66-amino-acid core and the CI N-terminal finger of region C of HEO were respectively replaced with the corresponding regions of hGR) activated the expression of MMTV-*CAT* but not of *vit-tk-CAT* (Fig. 1*a* and *b*). Conversely, HE61 (ref. 8) (in which the CII subregion of HEO was replaced with that of hGR) activated expression of *vit-tk-CAT*, but not of MMTV-*CAT*. To further delineate the amino acids responsible for this change of specificity, two new hER mutants were created in region CI of HEO. In HE73 (Fig. 1*b*), the N-terminal side (amino acids 185 to 188) and the finger tip (amino acids 189 to 201) were changed from the amino acids of hER to those of hGR, and in HE74 the C-terminal side amino acids 202 to 215 were changed (amino acid changes indicated by arrowheads in Fig. 1*b*; see also Fig. 4). HE73 retained the oestrogen-receptor (ER) specificity, whereas HE74 acquired the ability to activate MMTV-*CAT* but completely lost its capacity to activate *vit-tk-CAT* (Fig. 1*a* and *b*). Further mutagenesis demonstrated that the change in targ gene specificity observed with HE74 was essentially due to th replacement of three amino acids. Substitution of only thes three discriminating amino acids (HE82) causes a comple change of specificity (compare HE82, HE83, HE87, HE88 an HE89 in Fig. 1*a* and *b*).

To investigate the individual role of these three discriminatin residues, a series of single amino-acid substitutions was mad Each single substitution clearly decreased the efficiency of th corresponding proteins to act as ERs to a similar extent (HE8 HE85, HE86; Fig. 2*a* and *b*), but their target gene specificit was not changed. (Note, however, that HE84 slightly stimulate MMTV-*CAT*.) In contrast, a change in specificity was observe when substituting pairs of the discriminating amino acids wit the corresponding GR residues (HE90, HE91, HE92 in Fig. 2 and *b*). The two combinations that involve the Glu to Gl (E→G) substitution (HE90 and HE91) resulted in a loss of El function and a concomitant appearance of GR target gen specificity; this was particularly marked in HE91 (E, A→G, V) But HE92, in which the ER Glu203 was unchanged, recognize both ER and GR target genes, albeit with limited activatin capacity. Note that all receptor mutants were present in abou the same amount in the transfected HeLa cells, as judged fron western immunoblotting (Fig. 3 and data not shown), and tha the observed variations in reporter gene activation were therefor not due to differences in synthesis or stability of the mutate receptors.

Our results establish that three amino acids are of key import ance to the ability of the ER to discriminate between an ERE and a GRE. Interestingly, these three amino acids are no situated in the tip of the CI finger, but on its carboxyl side. I has been proposed that this side of the zinc finger may form ar





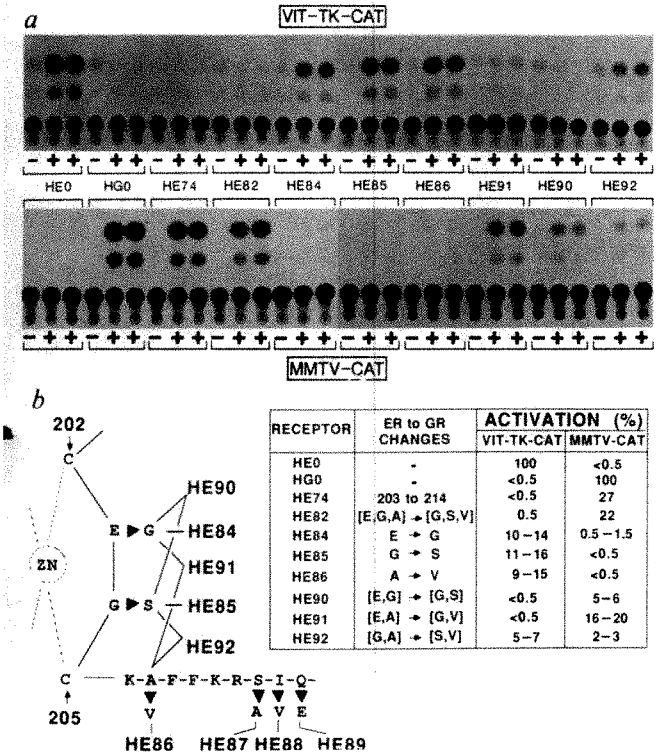
RECEPTOR	ACTIVATION (%) VIT-TK-CAT	MMTV-CAT
HE0	100	<0.5
HGO	<0.5	100
ER-GR·CAS	<0.5	26 – 32
HE61	15 – 18	<0.5
HE62	<0.5	15
HE73	67 – 80	<0.5
HE74	<0.5	27
HE82	0.5	22
HE83	52 – 81	<0.5
HE87	49 – 78	<0.5
HE88	55 – 75	<0.5
HE89	57 – 84	<0.5

FIG. 1 Target gene specificity of hER mutants containing hER to hGR amino-acid substitutions in the DNA-binding domain subregion CI. *a*, The expression vectors HEO (ref. 4), HGO (ref. 4), ER-GR.CAS (ref. 3), HE61 (ref. 8), HE62 (ref. 8), HE73, HE74, HE82, HE83, HE87, HE88 and HE89 were co-transfected into HeLa cells with either the oestrogen-responsive *vit-tk-CAT* or the glucocorticoid-responsive MMTV-*CAT* reporter genes. Oestradiol (10^{-8} M) was added when indicated (+) to transfections with HEO and its mutant derivatives and dexamethasone (10^{-7} M) was added to transfections with HGO. *b*, The CI region of hER (amino acids 185 to 215; one-letter terminology) with its potential 'zinc finger' is represented and the position of the second intron of the hER gene is indicated[7]. The positions of the amino-acid substitutions are indicated by an arrowhead pointing to the corresponding amino acid in hGR. The extent of the mutated region in each recombinant is defined by thin lines. Single average values (±20%) for the transcriptional activation of the two reporter genes (relative to either HEO or HGO, taken as 100%) result from three or more independent transfections with at least two different plasmid preparations. When two values are given, they correspond to two independent transfections with two different plasmid preparations. *Vit-tk-CAT* and MMTV-*CAT* expression were stimulated about 50- and 200-fold by HEO and HGO respectively.

METHODS. HeLa cells at ~40% confluence were co-transfected with 1 μg receptor expression vector, 1 μg *vit-tk-CAT* or MMTV-*CAT* reporter gene and 3 μg pCH110 (Pharmacia; a β-galactosidase expression vector used as a reference gene to normalize for variations in transfection efficiency). Transfection, preparation of cell extracts and quantitative determination of CAT activity have been described[8]. Mutagenesis of hER to generate HE73, HE74, HE82, HE83, HE87, HE88 and HE89 was essentially as described[8], except that we used hER cloned into the *Eco*RI site in pSG5 (ref. 19) instead of that in pSG1. The sequences of the synthetic oligonucleotides used for site-directed mutagenesis are available on request.

α-helix that interacts with the DNA major groove, so it could be responsible for specific recognition of the cognate DNA sequence (refs 8, 10, 11 and references therein). Indeed, a putative α-helix motif located on the carboxyl side of the zinc finger in the DNA-binding domain of the yeast LAC9 regulatory protein may be largely responsible for the binding specificity[12].

The consensus palindromic ERE and GRE, to which the corresponding receptors bind as dimers[5], differ by only two base pairs in each stem of the palindrome (ERE: TGACC versus GRE: TGTTC) (refs 1, 13, 14 and references therein). It is likely that the three amino acids characterized here are central to the discrimination between these two base pairs. Other amino acids conserved between the ER and GR CI subregion (see Fig. 4) are probably important either for specific interactions with the conserved bases of the palindromic elements or for non-specific interactions with the DNA backbone (see ref. 8 for a discussion of the possible role of the CII subregion in the overall efficiency of receptor binding to its responsive element (RE). That only two amino-acid substitutions (see mutant HE91 in Fig. 2) are required for the discrimination between two related REs, readily indicates that new receptors and REs might have co-evolved from their respective ancestors (note also the intermediate case of mutant HE92). Also, one would predict that nuclear receptors which share the same three discriminating amino acids should be able to activate the same RE. Comparison of the amino-acid sequences of eight nuclear receptors (see Fig. 4) shows that the amino-acid sequence of the carboxyl side of the CI finger is



RECEPTOR	ER to GR CHANGES	ACTIVATION (%) VIT-TK-CAT	MMTV-CAT
HE0	-	100	<0.5
HG0	-	<0.5	100
HE74	203 to 214	<0.5	27
HE82	[E,G,A] → [G,S,V]	0.5	22
HE84	E → G	10–14	0.5–1.5
HE85	G → S	11–16	<0.5
HE86	A → V	9–15	<0.5
HE90	[E,G] → [G,S]	<0.5	5–6
HE91	[E,A] → [G,V]	<0.5	16–20
HE92	[G,A] → [S,V]	5–7	2–3

FIG. 2 Target-gene specificity of hER mutants with amino acids 203, 204 and/or 207 changed to their GR homologues. *a*, Assay for CAT activity after transfection into HeLa cells of the expression vectors HE0, HG0, HE74, HE82, HE84, HE85, HE86, HE90, HE91 and HE92, together with either the *vit-tk-CAT* or MMTV-*CAT* reporter genes. Oestradiol and dexamethasone were added as described in Fig. 1*a* legend. *b*, The position of the amino-acid substitutions is as indicated in Fig. 1*b*. The transcriptional activation of the two reporter genes by the various hER mutants was expressed relative to HE0 and HG0, as indicated in Fig. 1*b*. Mutagenesis of hER to generate HE84, HE85, HE86, HE90, HE91 and HE92 and all other methods were performed as described in the legend to Fig. 1, except that cell-extract aliquots corresponding to five-times less β-galactosidase activity were used for CAT assays in the case of HE0 and HG0 transfections.

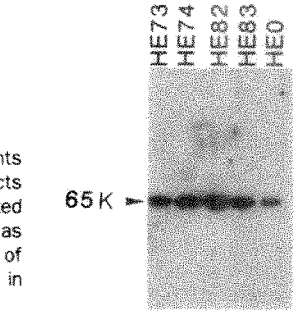


FIG. 3 Western blot analysis of hER mutants transiently expressed in HeLa cells. Extracts from HeLa cells were transfected as indicated and processed for immunoblot analysis as described[8]. The relative molecular mass of the receptor mutants is indicated in thousands.

remarkably conserved, with the striking exception of the three discriminating amino acids (indicated by arrows in Fig. 4); two subfamilies can be identified (Fig. 4*a* and *b*). The four members of one of these subfamilies (Fig. 4*a*) are known to recognize a common RE (refs 15, 16 and references therein). Moreover, it has recently been shown (ref. 17; H. de V., D. Metzger and P.C., manuscript in preparation) that two members of the second subfamily (Fig. 4*b*) which share the same three discriminating amino acids (retinoic acid receptor and thyroid hormone receptor, RAR and T3R respectively) may recognize a common palindromic RE, which is related to the ERE. (Considering the sequence similarities, it is probable that the RE of the vitamin D3 receptor is also close to that of RAR and T3R.) Interestingly, we have recently found (H. de V., D. Metzger and P.M., manuscript in preparation) that both T3R and RAR, which share two out of the three discriminating amino acids with the ER, can activate transcription of a reporter gene containing an ERE (but not a GRE), albeit with a lower efficiency than the ER (see also ref. 18 for an account of a weak stimulation of *vit-tk-CAT* by RAR). This observation is consistent with the properties of HE86, which contains the two discriminating amino acids common to ER, T3R and RAR, and specifically activates *vit-tk-CAT*, although with less efficiency than the wild-type hER (HE0) (Fig. 2).

Finally, it should be stressed that our data do not exclude the possibility that additional amino acids (such as some of those located in the finger tip) might help in the discrimination between an ERE and a GRE, although the amino acids we have identified here are sufficient. In this respect we note that HE82 is significantly less efficient than HG0 in the activation of MMTV-*CAT* (Fig. 1), and that its affinity for a GRE *in vitro* is lower than that of the GR (unpublished data). Additional

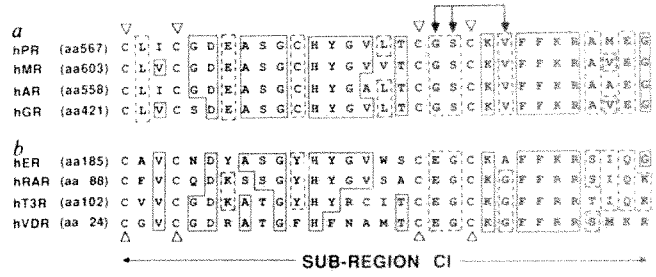


FIG. 4 Comparison of the amino-acid sequences of the subregion CI (N-terminal 'zinc finger') of the DNA-binding domain (region C) of various members of the human nuclear receptor family. The conserved cysteine residues that may tetrahydrally coordinate zinc to form a 'zinc finger' (see Fig. 1*b*) are indicated by open arrowheads. The arrows point to the position of the three 'discriminating' amino acids (see text). Amino acids which are common to only one of the two subfamilies (*a*, hPR, hMR, hAR and hGR; *b*, hER, hRAR, hT3R and hVDR; see text) are boxed with dashed lines, whereas those common to the two subfamilies are boxed with solid lines. The receptors hPR (ref. 20), hMR (ref. 16), hAR (ref. 21), hGR (ref. 22), hER (ref. 23), hRAR (refs 18 and 24), hT3R (ref. 25) and hVDR (ref. 26) are the progesterone, mineralocorticoid, androgen, glucocorticoid, oestrogen, retinoic acid, thyroid hormone and vitamin D3 receptors respectively.

mutagenesis aimed at identifying such possible helper amino acids is in progress. □

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Fertile transgenic rice plants regenerated from transformed protoplasts

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THE generation of transgenic plants using gene transfer techniques is important to both the investigation of gene regulation and the genetic engineering of crops[1]. The Ti plasmid of *Agrobacterium tumefaciens* is now routinely used to transform dicotyledonous plants[2], and the transfer of foreign genes to unorganized tissue[3–6] and plants[7,8] has been accomplished using direct DNA transfer methods[9–11]. A protocol for the easy and reproducible production of fertile transgenic cereals, however, has not yet been described. We report here the production of fertile transgenic rice plants obtained by introducing the bacterial *hph* gene, encoding hygromycin B resistance[12] (Hm^r), into protoplasts of *Oryza sativa* (L.) by electroporation. The non-selectable gene encoding β-glucuronidase was also transferred with the *hph* gene and its expression was detected in the progeny of the stable transformant.

The kanamycin resistance (Km^r) gene coding for neomycin phosphotransferase II (NPT II) is often used as a selectable marker to produce transgenic plants[1]. We found, however, that the bacterial *hph* gene is a more effective marker for the selection of stable transformants from embryogenic rice protoplasts and on the basis of this we chose, as an integration vector, plasmid pGL2, which carries the *hph* gene flanked by the 35S promoter and a polyadenylation site from cauliflower mosaic virus[13] (CaMV) (Fig. 2*a*).

Because embryogenic protoplasts of rice are small (10–20 μm in diameter), electroporation was carried out using a long pulse (10 ms), generated by a large capacitor (800 μF) and a moderate voltage (300 V cm^{-1}). Under these conditions, the plating efficiency (0.2–1.2%, Table 1) was 10–20% of that of non electroporated control protoplasts grown by the nurse cultur method[14]. No modification of the original protocol was require for the culture of electroporated protoplasts. After 2–3 week of selection wth hygromycin B (20 μg ml^{-1}), resistant colonie became clearly visible (Fig. 1*a*). Little background colon growth was observed in control experiments in which hygro mycin B (20 μg ml^{-1}) was added to the R2 medium[15] 2 week after electroporation.

The frequency of resulting Hm^r clones depended on th protoplast preparation and the cuvettes used for electroporatio (Table 1). In all the experiments, the transformation frequencie fell in the range 0.1–0.6% of clones subjected to selection. Thi range is lower than those obtained for the recovery of Km clones after the electroporation of maize protoplasts[5,8] and th treatment of non-embryogenic rice protoplasts with polyethyl ene glycol (PEG)[6].

Most of the Hm^r colonies continued to grow after transferrin them to a solid N6 medium[16] containing the same concentratio of hygromycin B. Three to four weeks later, the Hm^r calli were transferred to an N6 regeneration medium[14] containing nc hygromycin B. Within 3–6 weeks, shoots as well as roots became visible. The efficiency of regeneration is evident from the fac that more than 50% of the Hm^r calli yielded shoots in every experiment (Fig. 1*b*).

The presence of the *hph* gene on the chromosomes of stable transformants was shown by a Southern blot analysis of the plants regenerated from Hm^r calli (Fig. 2*a–c*). After the digestion of genomic DNA with *Bam*H1, six plants from four different experiments were found to contain a 1.1-kilobase (kb) fragment that hybridized to the *hph* gene (lanes 3–8). The detection of multiple bands, after digestion with *Eco*RV (lanes 9–11), indicated the presence of multiple chromosomal integration sites. By using a mixed probe containing both the *hph* probe and a single copy gene probe, the copy number of the integrated *hph* gene was estimated to be 2–10 per doploid cell (data not shown).

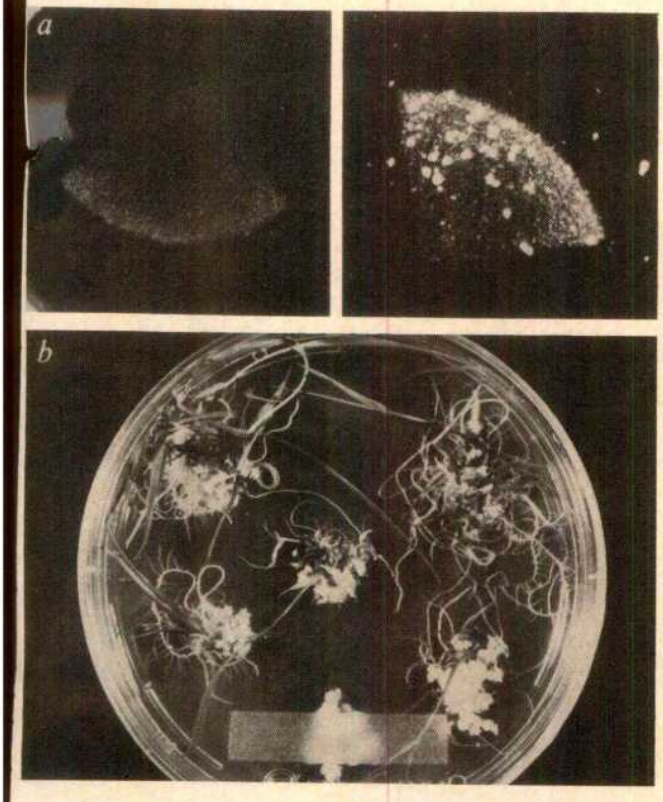
Plantlets regenerated from Hm^r calli were transferred to pots where they grew to maturity, flowered and set seeds (Fig. 1*c*). From fourteen such plants, we chose four, plants 3-1, 6-1, 6-3 and 8-1, to study the transmission of the *hph* gene. Thus, selfed seeds were subjected to hygromycin B at a concentration (30 μg ml^{-1}) previously found to be sufficient to inhibit the germination of control seeds. The subsequent survival of selfed seeds showed that the active *hph* gene had been transmitted to the progeny (Fig. 1*d*; Table 2).

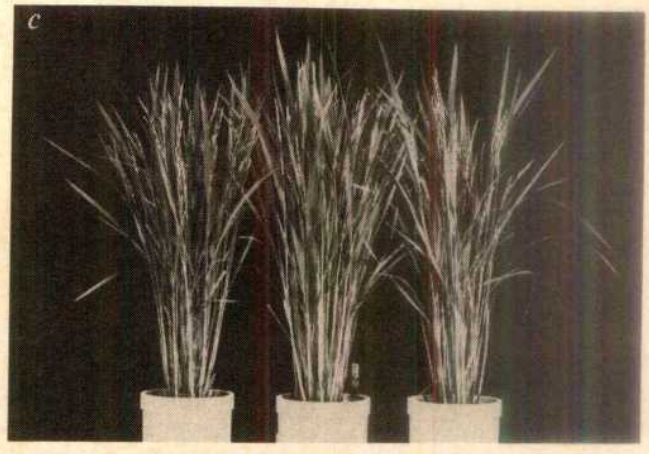
Further evidence for this transmission was obtained by germinating selfed seeds in the absence of hygromycin B and analysing their DNAs by Southern hybridization (Fig. 2*c*). Among ten progeny from the plant 6-3, seven contained the *hph* gene. Similarly, among nine progeny from the plant 8-1, six contained sequences hybridizing the *hph* gene. The two bands which hybridized the *hph* gene were found to co-segregate in the progeny of the plant 8-1. These results indicate that either a single insert or multiple inserts of the functional *hph* gene are present on the chromosomes of the primary transformants. To clarify the mode of transmission of the integrated *hph* gene, selfed and backcross progeny of other transformants are now being investigated.

TABLE 2 Transmission of the Hm^r phenotype to progeny

Seed	Total no. seeds tested	No. resistant seeds found	No. sensitive seeds detected
Control	96	0	96
3-1 self	40	38	2
6-1 self	95	85	10
6-3 self	29	25	4
8-1 self	45	31	14

The methods for testing the Hm^r phenotype are described in Fig. 1*d*.





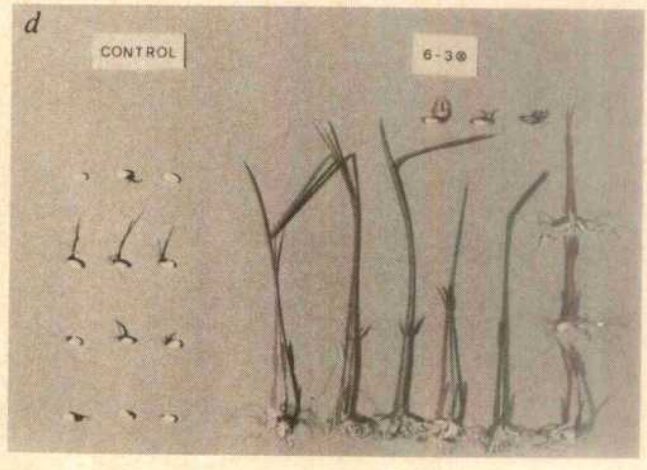
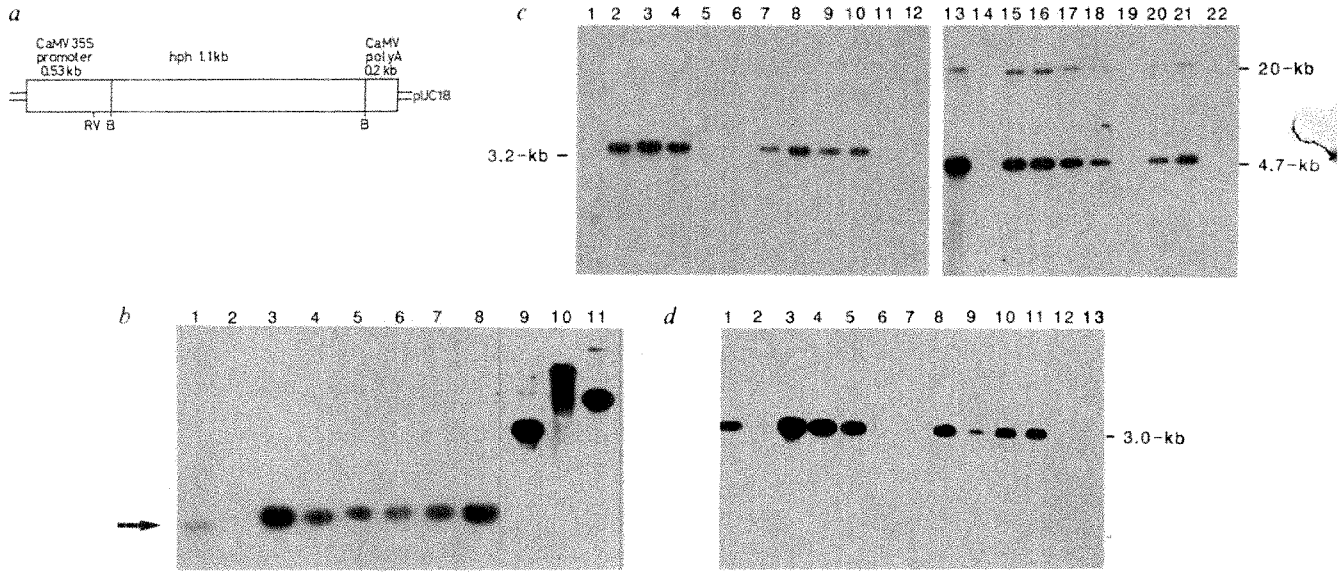


FIG. 1 Selection, plant regeneration and progeny analysis of transformants. *a*, Growing Hm^r colonies after 3 weeks of selection with 20 μg ml^{-1} hygromycin B (5 weeks after electroporation). Protoplasts were electroporated in the presence of plasmid DNA and carrier DNA (right); control culture electroporated with carrier DNA alone (left), *b*, Regeneration of shoots and roots from Hm^r calli, *c*, Fertile plants obtained from Hm^r calli. *d*, Test of the Hm^r phenotype in the control seeds (left) and the progeny of the transformant 6-3 (right). METHODS. Protoplasts were isolated from 1–2-month-old suspension cultures derived from mature embryo calli of *cv. Nipponbare* as described previously[14]. The protoplasts were washed twice and resuspended (4×10^6 protoplasts ml^{-1}) in a buffer containing 70 mM KCl, 5 mM $MgCl_2$, 0.1% MES, pH 5.8 and 0.4 M mannitol. Aliquots (0.5 ml) of the protoplast suspension were mixed with the supercoiled pGL2 plasmid DNA (10 μg ml^{-1}) and carrier DNA (50 μg ml^{-1}). Carrier DNA was prepared by dissolving calf thymus DNA (Sigma) in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 and sterilized using a disposable filter. After the incubation of the protoplast-DNA mixture in ice for 5 min, a sample (0.5 ml) was transferred to a precooled plastic cuvette and electroporated by a capacitor-discharge system (X-cell 450, Promega). After 10 min at 4 °C, protoplasts were mixed with 0.5 ml of the molten agar R2 medium[15] containing 2.5% agarose (Sea Plaque, FMC) in a plastic plate (35 mm in diameter). After cooling, the agarose was cut into four blocks, which were transferred to a plastic plate (6 cm in diameter) containing 5 ml of the R2 medium and cultured by the nurse culture method[14]. Hygromycin B (Sigma) was added to the medium 2 weeks later at a final concentration of 20 μg ml^{-1}. Agarose blocks containing growing colonies were transferred 2–3 weeks later to an N6 soft agar medium[16] containing 20 μg ml^{-1} hygromycin B and incubated for 1–2 weeks. Individual colonies were then transferred to the same N6 medium with increased agarose concentration (0.5%). When the calli were ~5 mm in diameter, they were transferred to the regeneration medium. Plant regeneration was as described previously[14]. For analysis of Hm^r in progeny, selfed seeds were sterilized in 0.6% $NaClO_4$, washed twice in sterile distilled water and placed onto MS medium[19] containing 30 μg ml^{-1} hygromycin B. They were incubated for 2 weeks at 25 °C under light.

TABLE 1 Frequency of Hm^r clones

Experiment	Cuvette	pGL2 (μg ml^{-1})	No. protoplasts used ($\times 10^6$)	No. clones tested for Hm^r ($\times 10^3$)	No. Hm^r clones found	Frequency of stable transformations (%)
I	1	0	2	10.5	0	0
	2	10	2	14.3	65	0.45
	3	10	2	14.5	58	0.40
	4	10	2	11.6	75	0.65
	5	10	2	13.7	70	0.50
	6	10	2	12.4	80	0.60
II	1	0	2	17.8	0	0
	2	10	2	24.0	32	0.13
	3	10	2	18.0	27	0.15
	4	10	2	4.8	12	0.25

Experiments I and II consisted of several electroporation trials, each of which was performed with a cuvette, using protoplasts derived from one preparation. Colonies subjected to selection for Hm^r were counted 12 days after electroporation. The number of Hm^r clones was scored after 3 weeks of culture in the presence of 20 μg ml^{-1} hygromycin B.



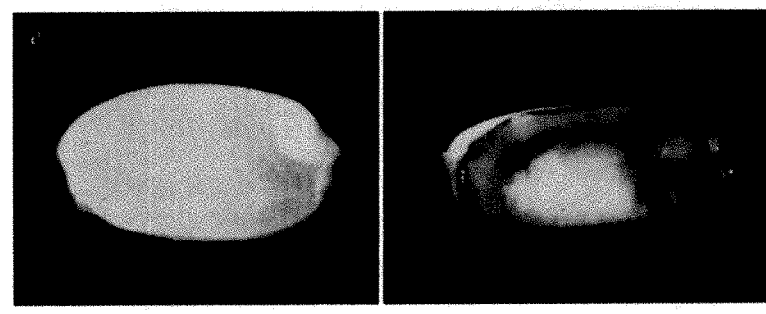


FIG. 2 Molecular confirmation of the chromosomal integration of the foreign genes. *a*, Diagram of pGL2. pGL2 is a pUC18 derivative carrying the 35S promoter and a polyadenylation site of CaMV (ref. 13) together with the *Bam*H1 segment containing the *hph* gene derived from pLG88 (ref. 20). It contains a unique *Eco*RV site within the 35S promoter region: B, *Bam*H1; RV, *Eco*RV. *b–d*, Southern blot analysis of the genomic DNA from primary transformants and their progeny. *b*, Genomic DNA from nine independent Hm^r plants digested with *Bam*H1 or *Eco*RV. Lane 1, 1.1-kb *Bam*H1 fragment of pGL2 containing the *hph* gene indicated by arrow; lane 2, DNA from non-transformed plants; lane 3–8, *Bam*H1-digested DNA from 6 independent Hm^r plants; lane 9–11, *Eco*RV-digested DNA from three Hm^r plants. *c*, DNA from progeny of transformants 6-3 and 8-1 digested with *Eco*RV. Lane 1, DNA from non-transformed plants; lane 2, DNA from the transformant 6-3; lanes 3–12, DNA from 10 progeny of the transformant 6-3; lane 13, DNA from the transformant 8-1; lanes 14–22, DNA from nine progeny of the plant 8-1. *d*, DNA from the GUS transformant G1 and its progeny digested with *Eco*RI and *Hin*dIII. Lane 1, pBI221 digested with *Eco*RI and *Hin*dIII,; lane 2, DNA from non-transformed plants; lane 3, DNA from the primary transformant G1; lanes 4–13, DNA from 10 progeny. *e*, Expression in the seed of the GUS marker co-transformed with pGL2. Left, control seed; right, progeny seed expressing GUS activity.

METHODS. DNA samples were prepared from transgenic plants according to a published protocol[21]. For Southern blot analysis[22], 1–2 μg of digested DNA was electrophoresed in a 1.0% agarose gel, transferred to a nylon membrane (Bio-Rad) and the blots were hybridized with the ^{32}P-labelled[23] 1.1-kb *Bam*H1 fragment of pGL2 corresponding to the *hph* gene (*b*, *c*) or 1.9-kb *Xba*I-*Sst*I fragment of pBI221 corresponding to the GUS gene (*d*). The *Bam*H1 fragment of pGL2 (10 pg) (*a*) or 12 pg of pBI221 digested with *Eco*RI and *Hin*dIII (*c*) was electrophoresed as a marker. For cotransformation of the GUS marker with the *hph* gene, 20 μg ml^{-1} of pBI221 was mixed with 10 μg ml^{-1} of pGL2 and 50 μg ml^{-1} of carrier DNA and electroporated as described in Fig. 1. For histochemical staining of the GUS activity, seeds were cut into halves and stained with X-gluc according to ref. 24.

A preliminary experiment to examine co-transformation of an unlinked, non-selectable gene was performed using the β-glucuronidase (GUS) gene under the control of the CaMV 35S promoter and the 3′ region of the nopaline synthetase gene[17]. The addition of this GUS plasmid (20 μg ml^{-1}) did not influence the plating efficiency or the frequency of Hm^r colonies. The selfed seeds of one of the Hm^r plants that showed GUS activity in their leaf extracts, referred to as G1, were subjected to further analyses. Southern blot analysis of G1 and its progeny showed that 7 out of 10 progeny plants contained the complete 3.0-kb GUS gene (Fig. 2*d*). The progeny seeds were individually cut into two halves and stained with 5-bromo-4-chloro-3-indolylglucuronide (X-gluc) to detect GUS activity. Whereas no activity was detected in 20 untransformed control seeds, 41 out of 57 transformed seeds were found to have GUS activity in the embryo and the endosperm (Fig. 2*e*), indicating that the GUS gene, integrated in the primary transformant, is stably inherited by progeny.

These results demonstrate that the co-transformation of a non-selectable gene with a selectable marker can be used to generate fertile transgenic rice. It seems that the construction of a plasmid carrying both the selectable marker and the non-selectable gene is not a prerequisite for the successful transfer of the latter, and that, as observed in the case of tobacco[18], co-transformation is a simple alternative method for generating transgenic plants carrying active genes. □

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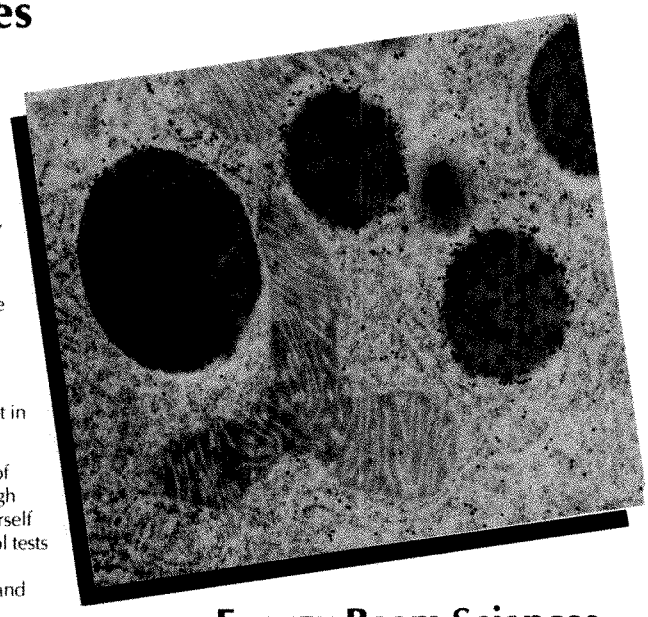
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Genomic footprinting with *Taq* polymerase

H. P. Saluz and J. P. Jost

The linear amplification of chemically sequenced DNA with DNA polymerase from *Thermus aquaticus* permits the direct detection of DNA methylation and protein–DNA interaction sites *in vivo*.

DNA methylation and protein–DNA interactions play a crucial role in gene regulation[1,2]. Genomic sequencing — pioneered by Church and Gilbert[3], and further refined in our laboratory[4] — enables the study of the methylation state of any gene in a strand- and sequence-specific manner.

When used to investigate areas of protein/DNA interaction, the technique is referred to as genomic footprinting. In this technique, cells in suspension are treated with dimethylsulphate (DMS) or ultraviolet light to modify specific DNA bases, which undergo a quantitative cleavage reaction (β-elimination)[5–7]. The modified sites are then identified by genomic sequencing. Proteins bound to DNA protect these areas from modification by DMS and subsequent chemical cleavage, leaving their "footprint" on the DNA. In contrast, ultraviolet light cleaves at sites of protein–DNA interaction.

Despite the potential of *in vivo* footprinting for revealing protein–DNA interactions and genomic sequencing for detecting modifications to the DNA's native state, the current procedure is rather time-consuming and complex. We sought therefore to simplify the technique and make it more accessible to less experienced users[8]. A flow diagram of our new method is shown in Fig. 1*a*.

Abbreviated strategy

The first steps of both the old method and our new technique are the same: total genomic DNA is digested with a suitable restriction enzyme, and the resulting DNA fragments are chemically sequenced as described by Maxam and Gilbert[9], Fritzsche *et al.*[10], or Rubin and Schmid[11]. But in our technique, sequencing is followed by selective, linear amplification with the thermostable DNA polymerase from *Thermus aquaticus*, using a synthetic primer labelled to a very high specific radioactivity. The products of the linear amplification (Fig. 2) are then run on a sequencing gel, and the sequence information is directly obtained by exposing the dried gel to an X-ray film.

A step forward

The new technique permits sequence information to be gathered for any gene, regardless of its copy number and the size of the total genome, without electroblotting or hybridization steps (Fig. 1 *b*). Moreover, as restriction enzymes are used only to reduce the viscosity of the genomic

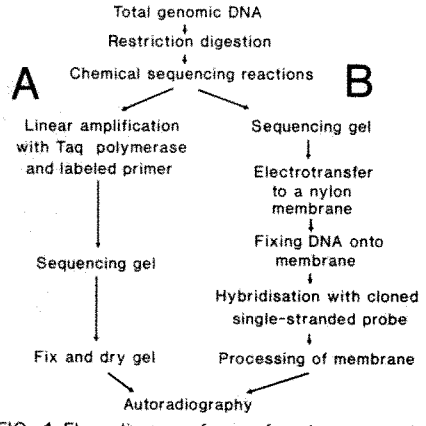


FIG. 1 Flow diagram for performing genomic experiments using the new expedited procedure (*a*), and the classical method of genomic sequencing (*b*).

DNA in the reaction mixture, less expensive endonucleases can be used, as long as they do not cleave within the target sequence.

But the success of our technique is highly dependent upon the purity of the *Taq* polymerase used, which can vary from batch to batch. The recent commercial availability of recombinant *Taq* polymerase should eliminate this problem. [Editor's note: Recombinantly produced *Taq* polymerase can be purchased from Perkin-Elmer Cetus and United States Biochemical Corporation. Purified natural *Taq* is sold by Perkin-Elmer Cetus, Stratagene, BRL, Beckman Instruments, and Promega.]

Our simplified procedure for studying the native state of genomic DNA relies on the linear amplification of cleaved DNA

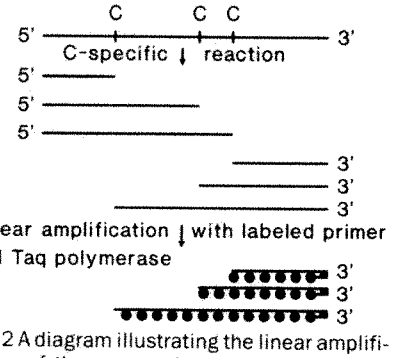


FIG. 2 A diagram illustrating the linear amplification of the sequencing reaction products. Restriction enzyme-digested DNA is first subjected to chemical sequencing reactions. The fragments generated by β-elimination are then annealed to labelled oligonucleotide primers (black squares), and the complementary strand (dotted line) is synthesized by *Taq* polymerase.

fragments to produce many radioactively labelled copies. The resulting increase in sensitivity allows the more rapid detection of single-copy mammalian genes, and should permit the investigation of such complex genomes as those of higher plants. The technique retains all of the benefits of the original genomic sequencing procedure. The unique thermostability of *Taq* polymerase permits the optimal annealing of the primer to the denatured target template, and its faithful elongation. But so far, procedures for studying DNA methylation are still based on the detection of chemical cleavage products. 5-Methylcytosine reacts poorly with hydrazine, resulting in a lack of cleavage upon alkaline treatment. The position of the 5-methylcytosine is then revealed by a gap in the sequence ladder.

Because chemical cleavage of the DNA results in fragments of different lengths, it is only possible to amplify each fragment linearly. But if procedures were available quantitatively to deaminate 5-methylcytosine residues to produce thymidine, the modified DNA could be amplified exponentially by the popularly used symmetrical amplification procedure of the polymerase chain reaction (PCR)[12,13]. Following dideoxy sequencing, the position of the original 5-methylcytosine would be detected by the presence of an A-T pair instead of a G-C pair in the undeaminated control DNA. We are now investigating this approach, because it would speed up the procedure enormously. □

Hanspeter Saluz and Jean-Pierre Jost are at the Friedrich Miescher-Institut, PO Box 2543, CH-4002, Basel, Switzerland. For more information, fill in reader service number 100.

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FASEB exposition in New Orleans

The best of what biology has to offer will be on display at next week's FASEB meeting in New Orleans, Louisiana — from a sponge for destaining electrophoresis gels to transgenic mice for cancer research.

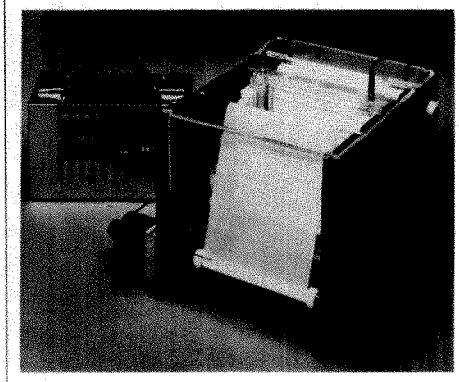
KONTRON Instruments sells an **ultracentrifuge toolkit** that contains everything needed for the correct use of Kontron ultracentifugation rotors, tubes and capping systems — packaged together in a black storage case (*Reader Service No. 101*). The toolkit includes a tube cap vise with inserts; tube, cap and adaptor removal tools; a torque wrench with sockets; a hex driver; various lubricants; and tube stands in a range of sizes. Kontron says the tools can extend the lifetime of ultracentrifuge rotors and capping systems, if used properly. The company also offers IBM-compatible centrifugal separation simulation software which allows users to predict the optimal conditions for performing a variety of separations using any standard rotor.

DuPont's transgenic OncoMouse — the world's first patented animal — is now on sale for use in cancer research (*Reader Service No. 102*). The **transgenic mouse** is reared by Charles River Laboratories, and contains the viral Harvey *ras* (v-Ha-*ras*) oncogene controlled by the mouse mammary tumour virus (MMTV) promoter. OncoMouse is intended to be used to study the pathogenesis of cancer, to screen chemopreventive and anticancer agents, and to test the carcinogenicity of compounds: 30 per cent of female mice develop breast cancer within 100 days. Both males and females develop parotid adenocarcinomas and harderian hyperplasia. DuPont also sells a line of monoclonals specific for the p21 protein product of v-Ha-*ras* for measuring oncogene expression. The price of each OncoMouse is $50 (US) for orders under 25. The company plans to offer future strains of OncoMouse incorporating rat*neu* and c-*myc* oncogenes, under the control of other types of promoters.

Gel goodies

ICN Biomedicals has introduced an **electrophoresis destaining sponge** for leaching Coomassie Brilliant Blue stains out of electrophoresis gels (*Reader Service No. 103*). ICN Biomedicals says the sponge's high affinity for Coomassie reduces by half the number of methanol rinses required for destaining. The 5 × 5 cm sponges are supplied in packs of 50. The company says one sponge is sufficient for destaining a 10 × 10 × 0.1 cm gel, with no changes in the standard destaining procedure.

Hoefer Scientific has a new **sequencing apparatus** that separates and blots fragments in one operation (*Reader Service No. 104*). The model TE2000 direct blotting sequencer first separates fragments on a 14 × 16 cm gel. Fragments then migrate off the bottom of the gel onto a nylon membrane moving on a belt directly beneath the gel. The speed of the belt is adjustable, to eliminate the band crowding often seen at the top of sequencing gel blots. Hoefer says that the new sequencer/blotter can be used to sequence up to 400 bases in a single run, without handling fragile gels. The membrane can also be used to detect sequences by colourimetric detection, eliminating the need for working with radioactive isotopes.



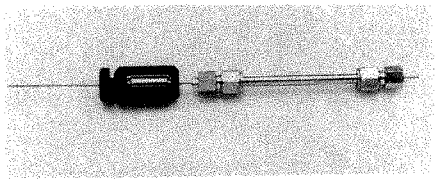
Up to 400 bases-a-run: Hoefer's TE2000.

AT Biochem recently unveiled a new **electrophoresis polymer** as an alternative to agarose and polyacrylamide (*Reader Service No. 105*). The company will not disclose the composition of its Hydrolink polymer, pending approval of its patent. But AT Biochem says the Hydrolink matrix is less susceptible to breakage, produces sharper bands, is less toxic, and can accommodate ten times the loading levels of traditional gel media. The company sells formulations for both double-stranded DNA separations and sequencing gels. The Hydrolink media comes as a ready-to-pour liquid, with specially formulated buffers and denaturing reagents that eliminate the need for urea. The company plans to introduce a matrix for protein separation later this year.

Chromatographic analysers

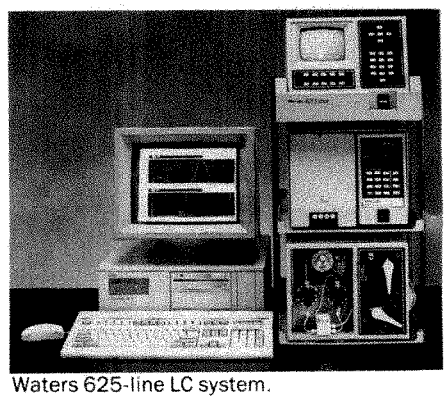
Applied Biosystems has introduced an **amino acid analyser** that combines hydrolysis, derivatization and separation steps in one complete system (*Reader Service No. 106*). The model 420A amino acid analyser's hydrolyser unit breaks the bonds between amino acids to liberate free amino acids using hydrochloric acid at temperatures of greater than 1,000 °C. The unit completely eliminates the need for time-consuming manual hydrolysis: researchers simply add the protein sample and return in less than an hour to collect results. The 420A's PTC derivatization unit labels free amino acids with a chemical which Applied Biosystems says allows detection at high sensitivities. Once derivatized, the amino acids are separated by liquid chromatography and detected and identified by comparison with a known standard.

Hamilton has expanded its range of **reversed-phase columns** to include columns especially designed for analysis in protein and peptide matrices (*Reader Service No. 107*). Hamilton's new PRP-3 columns can be regenerated in an overnight process in order to eliminate residual protein buildup and to restore performance. The PRP-3 columns are available in pre-column, analytical and preparative sizes, and can be used over the pH range 1–12.



The Hamilton PRP-3: acid to alkiline.

Waters recently introduced a liquid chromatography system designed specifically for bioseparations (*Reader Service No. 108*). The Waters model 625 **bioseparation chromatography system** incorporates non-metallic polymeric materials with low dispersion capabilities for microbore, analytical and micropreparative separations. The system can be used with ion exchange, affinity, hydrophobic interaction, gel filtration and reverse phase separations relevant to the biochemist, and accommodates both photodiode array and tunable UV/visible detection.



Waters 625-line LC system.

Waters says the system has a 0.01 to 5 ml min^{-1} flow range capability and a 4,000 p.s.i. operating limit. Special features include an automated plunger wash, four-buffer blending, flexible non-metallic tubing and injector, and finger-tightened fittings. The $19,000–29,500 (US) model 625 also provides Waters' PowerLine single point control of all gradient, injection, detection and external event parameters, with or without a computer.

Eldex has a new integrated, programmable **solvent delivery system** for applications ranging from microbore HPLC separations to high-resolution preparative-scale purifications (*Reader Service No. 109*). Eldex's model 9600 solvent delivery system features interactive menus for methods development without the need for programming skills. The unit will generate ternary gradients with pulse-free solvent delivery, and has input and output ports for interfacing with other chromatography peripherals. Eldex sells the instrument for $6,995 (US).



Programmable solvent delivery from Eldex.

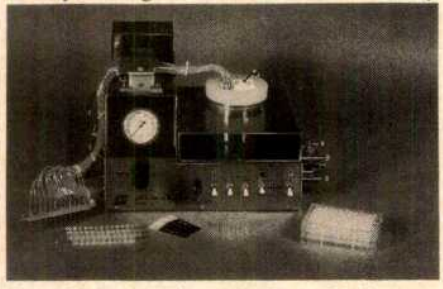
Nucleotide news

British Bio-technology Ltd sells a line of **DNA probes for interleukins** by the name of Designer Probes, after its successful line of Designer Genes (*Reader Service No. 110*). The Designer Probes recognize certain unique exons of genes coding for interleukins, and their mRNA. The probes are available for three or more exons within the genes for interleukin-1α, interleukin-1β, and interleukins 2–6. Probe cocktails consisting of an equimolar mixture of exon-specific probes are also available, to detect different regions of a gene simultaneously, yielding higher sensitivity. British Bio-technology Ltd also has a new **cytokine sampler kit** which contains trial-size samples of recombinant human cytokines interleukin-1α, -1β, -2, -3, -4, -6, and tumour necrosis factors α and β. The cytokines are produced from BBL's Designer Genes by R + D Systems in the United States. They are distributed in North and South America by R + D Systems, and in Japan by Funakoshi Pharmaceutical.

Version 5.0 of Hitachi's HIBIO DNASIS and PROSIS **sequence-manipulation software** has just been released (*Reader Service No. 111*). The new versions can perform homology searches three times faster than older releases, can create DXF-formatted output files, and can support HP Laserjet printers. DNASIS now contains two completely new analytical functions: protein coding region prediction based on Fikett's method; and secondary structure, nucleic acid sequence prediction based on Zuker's method. Restriction maps can now be displayed in circular and linear formats, together on the screen with the fragment, its size and map location. PROSIS now supports the parameter set for Rose and Roy hydrophilicity/hydrophobicity analyses, and can display Edmundson Wheel vectors of hydrophobic sites within a sequence. PROSIS and DNASIS cost $1,600 (US) each; current users can upgrade their versions for $400 (US) each.

New in immunology

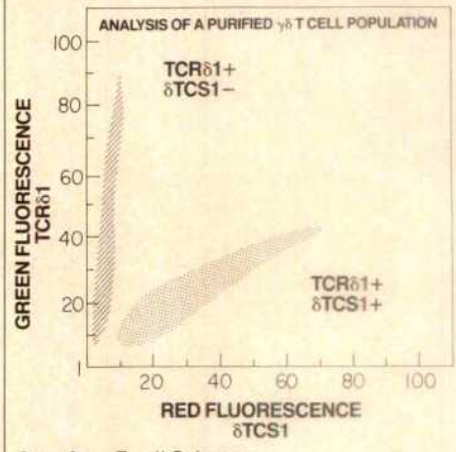
Dynatech Laboratories has a new 12-channel **dispensing system** for filling microtitre plates, slides, cards, strips and vials — the Quick Spense IIIe (*Reader Service No. 112*). Built by Sandy Spring Instruments, the Quick Spense IIIe is an automated liquid delivery system which couples time pressure dispensing with precision, cam-activated valving. Up to three reagents can be dispensed simultaneously, using 1–12 channels. Delivery volumes range from 5 μl to over 1 ml, and can be extended using an optional module. The dispensing head may be custom designed for any multiple dispensing format, and manual dispensing can be controlled by the use of the included foot switch or by the activation switch mounted on the control panel. Product contact parts can be flushed in place, or removed and autoclaved. Dynatech says the system has an accuracy rate of approximately 1.5 per cent for materials of standard viscosity.



Quick Spense IIIe for multichannel delivery.

Human **monoclonal antibody against HIV** is now available from the Swedish company BioInvent (*Reader Service No. 113*). The company develops the antibody through its patented *in vitro* immunization technology, which involves fusing peripheral lymphocytes isolated from a healthy blood donor which have been exposed to a recombinant immunodominant peptide from gp 41 to an EBV-infected hetero-myeloma. The resulting monoclonal is an IgM-kappa isotope antibody which BioInvent says has no cross-reactivity with unrelated proteins. BioInvent also offers human monoclonal antibodies specific for digoxin and cytomegalovirus.

T Cell Sciences has announced its second monoclonal antibody to the delta chain of the human T cell antigen receptor

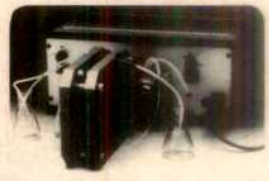


Data from T cell Sciences.

— Identi-T TCR61 (*Reader Service No. 114*). T Cell Sciences says the **T cell antigen receptor MAb** identifies a framework

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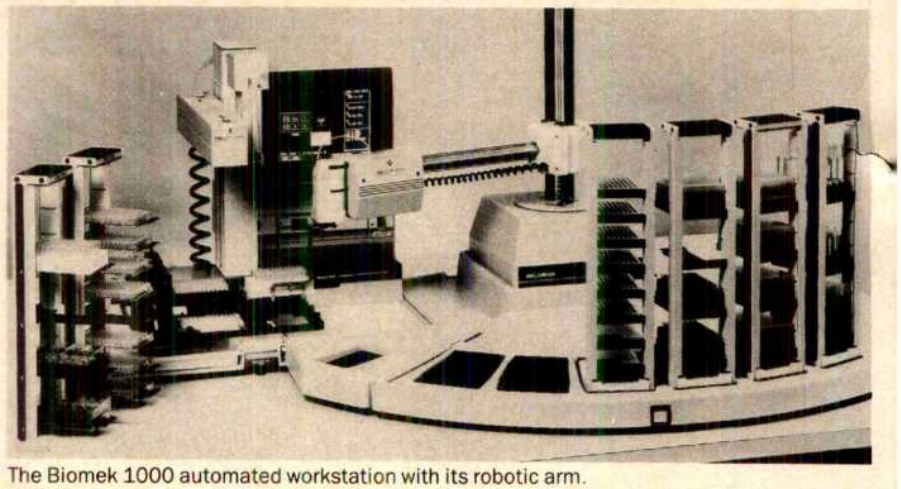
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Reader Service No.42



Identi-T FI antibody in action.

or common determinant of the delta chain as expressed on the surface of gamma-delta positive peripheral blood lymphocytes, interleukin-2 cultured thymocytes, specific T cell lines and in tissue sections. Identi-T TCR61 is available in purified, FITC conjugated and biotinylated forms for use in immunofluorescent staining of live cells, immunochemical staining of frozen tissue sections and fixed cells, and immunoprecipitation. T Cell Sciences also sells a monoclonal specific for the alpha chain of the T cell antigen receptor. Indenti-T αF1 binds to a nonpolymorphic determinant of the alpha chain, normally hidden on viable cells but exposed on fixed cells and frozen tissue sections. It also binds to T cell antigen receptor alpha-beta heterodimers. Identi-T αF1 offers an alternative to mRNA detection for studying the ontogeny of T cells, and can be used in Western blot analyses.



The Biomek 1000 automated workstation with its robotic arm.

Beckman Instruments' Biomek 1000 **automated laboratory workstation** can be configured for receptor binding studies (*Reader Service No. 115*). The $26,500 (US) Biomek SL robotic arm can be used for loading and unloading labware onto the $24,000 (US) Biomek 1000 system. The system includes wedge-shaped stack areas which hold up to six stacks of labware — such as pipette tips, multiwell plates and tubes — within reach of the robotic arm. The workstation automatically performs pipetting, plate washing and photometry readings. For receptor binding studies, Beckman also offers 2-ml Cube 2ubes in a 96-well format, and deep-well 96-well microtitre plates with a single well capacity of 1 ml. Both Cube 2ubes and deep-well microtitre plates come sterile or unsterile, with or without covers.

GIBCO Laboratories has a new **protein-free hybridoma medium** to be used as an alternative to serum-supplemented media for monoclonal antibody production (*Reader Service No. 116*). GIBCO says its PFHM-II medium, available as powder or liquid, contains no polypeptide growth factors, attachment factors, or mediators to complicate downstream purification, and that it performs as well as serum-supplemented media in conventional and high-density culture systems.

The literature shelf

Schleicher & Schuell's new four-colour **life sciences catalogue** describes apparatus and filter media available from the company for a variety of separation techniques, transfer methods, solid phase assays and related procedures (*Reader Service No. 117*). Among the new products featured are Schleicher & Schuell's Profile mini-electrophoresis system, Elutrap electroseparation unit, and dual-filter Uniflo Plus syringe filter unit. A section devoted to transfer media describes new Optibind reinforced nitrocellulose membranes, and a new section outlines the company's services for developers of test kits and device manufacturers. The free catalogue also includes a selection guide to help users choose the appropriate product for a specific application.

The new 1989 catalogue on **cell culture reagents** from Boehringer Mannheim Biochemicals features the company's Nutridoma media supplements, cell culture factors and cytokines, antibodies to factors and cytokines, dissociating and harvesting enzymes, hybridoma reagents, antibiotics and complementary products (*Reader Service No. 118*). The free catalogue outlines the form, purity, concentration, price and other pertinent information on the full range of Boehringer Mannheim's products for cell culture.

Stratagene's new **1989 catalogue** should hit the streets this month (*Reader Service No. 119*). The company's free catalogue will outline its Stratalinker for crosslinking nucleotides to membranes, its ThermalBase kit for DNA sequencing using *Taq* polymerase, and its Zap cDNA synthesis kit for uni-directional cloning. Also described will be Stratagene's custom library preparation services, its large selection of restriction and modifying enzymes, and its Epicurean Coli high efficiency competent *Escherichia coli*.

Setting it straight

The instrument for using caged neurotransmitters described in the 9 February issue (*Nature* **337**, 583; 1989) was developed while the author was at the School of Applied and Engineering Physics, Cornell University. Jeffrey Marque is now with the Spinco Division of Beckman Instruments. The article also should have referred to work on the measurement of light-activated neurotransmitters by H. A. Lester and J. M. Norbonne (*A. Rev. biophys. Bioengng* **11**, 151–175, 1982). □

These notes are compiled by Carol Ezzell from information compiled by the manufacturers. To obtain further details, fill out the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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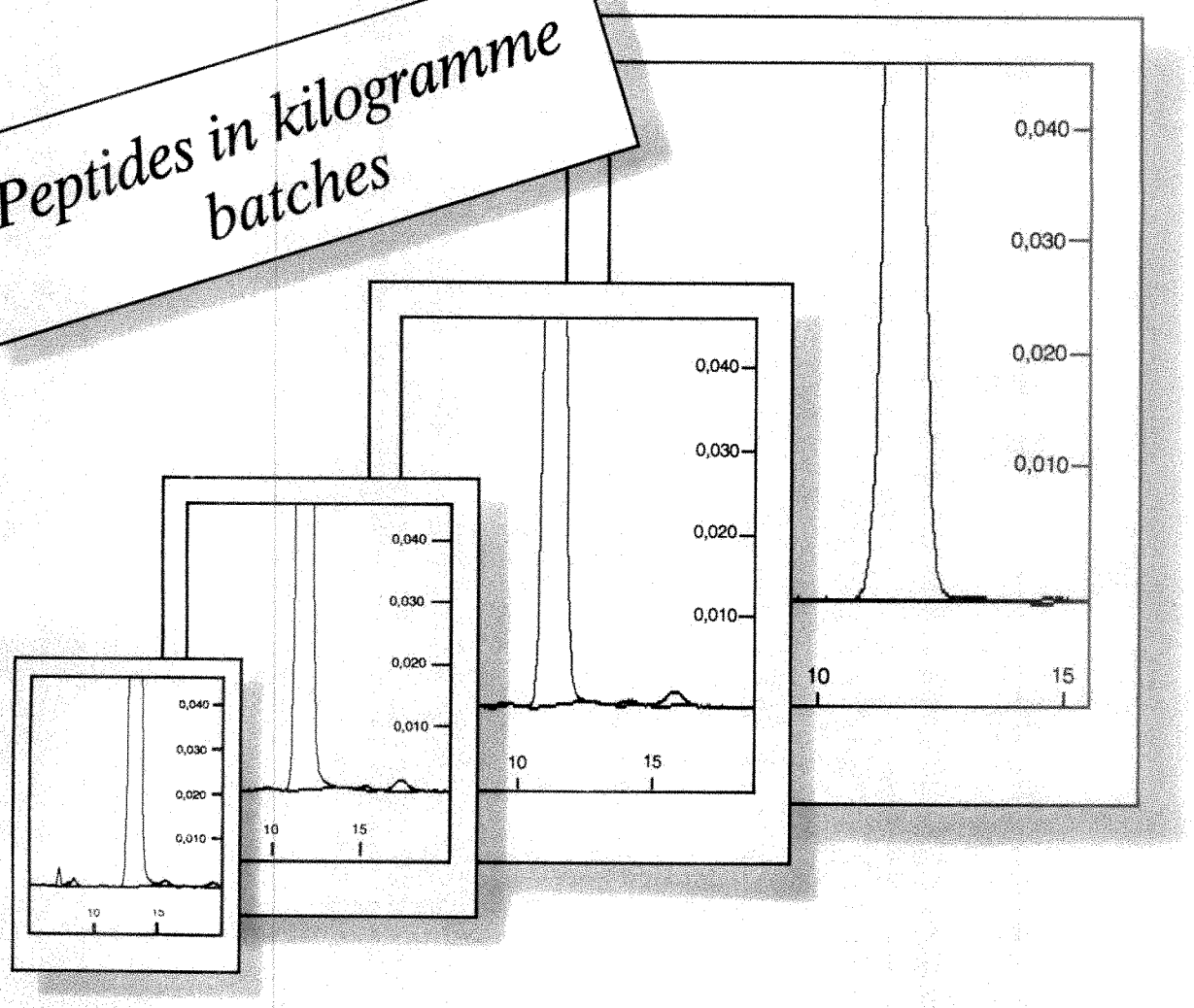
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Research assistants are also needed in the above areas as well as protein and protease chemistry and molecular biology. Requires B.S. or M.S. in related field preferably with 1–2 years experience.

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Applicants must submit curriculum vitae, summary of research interests and names of three references to: **Dr. Michael P. Czech, Director or Dr. John L. Sullivan, Search Committee Chairman, Program in Molecular Medicine, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655.**

An Equal Opportunity/Affirmative Action Employer (NW3466)A

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Dr. M.A.O. Siddiqui
Professor and Chairman
Department Anatomy & Cell Biology
SUNY Health Science Center at Brooklyn
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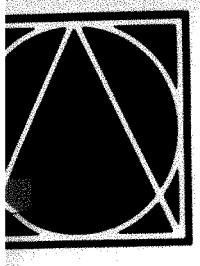
UNIVERSITE D'OTTAWA, Département de Biochimie

Le Département de Biochimie est à la recherche de **trois professeurs adjoints** avec possibilité de permanance pour entrer en fonction le 1er septembre, 1989. La disponibilité des postes est soumise à l'approbation budgétaire. Un doctorat est de rigueur. Les candidat(e)s choisi(e)s devront enseigner la biochimie et en français et an anglais au niveau pré-diplomé. Ils (elles) seront tenus(e)s d'entreprendre des programmes de recherche indépendante y compris la formation de candidats aux grades supérieurs. L'Université a une politique d'égalité en matière d'emploi. Les citoyen(ne)s canadien(ne)s ou résident(e)s permanent(e)s reçu(e)s sont invité(e)s à poser leur candidature en s'addressant au **Dr. P. Anderson, Département de Biochimie, Université d'Ottawa, Ottawa, Ontario, Canada K1H 8M5.**

UNIVERSITY OF OTTAWA, Department of Biochemistry

Applications are invited for **three assistant professor positions**, with the possibility of tenure. The positions are subject to budgetary approval and would begin Sept. 1, 1989. Ph.D. and evidence of research potential required. Candidates must be able to teach in English and French at the undergraduate level and be prepared to develop an independent research program including graduate student supervision. Employment equity is University policy. Canadian citizens and permanent residents of Canada are invited to apply to **Dr. P. Anderson, Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.**

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Both SK&F developed software (COSMIC/ASTRAL) and a wide range of standard packages, including SYBYL, are currently in use. In-house access to the Cambridge and Brookhaven data bases and various reaction and sequence data bases is also available.

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A competitive salary will be offered, and benefits will include a company car, performance-related bonus, BUPA and relocation expenses where appropriate.

Please write in confidence, enclosing a detailed CV and quoting reference no 33/89. to: Dr. T. R. Hutton, Personnel Manager, Smith Kline & French Research Limited., The Frythe, Welwyn, Hertfordshire AL6 9AR.

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University of Birmingham
School of Basic Medical Sciences
Lectureship in Physiology

A vacancy exists for a lecturer with an established research record in cardiovascular physiology. We are seeking a scientist with a special interest in cardiac and vascular factors limiting performance but persons with expertise in other areas of cardiovascular physiology are encouraged to apply.

The main research areas in the Department are: cardiovascular control, neurophysiology of motor and sensory system, cellular neurophysiology and renal physiology.

The Department is responsible for teaching physiology to medical, dental and science students.

Salary in the range £9,260-£19,310 per annum, plus superannuation.

Informal enquiries can be made to Professor J.H. Coote, Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TJ (Tel. 021-414-6915/6).

Further particulars and application forms may be obtained from the **Senior Assistant Registrar, University of Birmingham Medical School, Vincent Drive, Birmingham B15 2TJ (Tel. 021-414-4049)** to whom applications, including c.v. and the names and addresses of three referees, should be sent by not later than April 10th 1989.

AN EQUAL OPPORTUNITIES EMPLOYER (8759)A

DEPARTMENT OF VISUAL SCIENCE,
Institute of Ophthalmology,
Judd Street,
London, WC1H 9QS

Senior Lecturer/Lecturer

Candidates with a proven record in research in visual neurobiology, visual psychophysics or scientific topics relevant to investigative ophthalmology will be considered. The Department has a rapidly expanding externally funded research programme and with recent new appointments to staff, provides an ideal environment for those wishing to develop an independent career in vision research in the UK. The starting date is negotiable. Salary within the scale £15,105–£19,310 or £20,270–£22,910 p.a. plus £1,650 London Allowance.

Postdoctoral Research Fellow

We have two vacancies for candidates with an interest in pursuing a career in visual neurobiology. One project involves in vitro intracellular recording techniques and is concerned with excitatory amino acid receptors in visual transmission, but will also provide opportunity for in vivo work on the same system. This is funded for five years. The other, funded for two years in the first instance, is to support either anatomical or electrophysiological studies on feedforward and feedback projections in the central visual system. The starting date is 1st Oct 1989 or sooner if appropriate. Salary will be in the range £9,865–£14,500 p.a. according to age and experience, plus £1,650 London Allowance.

Applicants should send a full curriculum vitae with names of two referees to Professor A.M. Sillito at the address above from whom further details can be obtained (01–387–9621 x258) (8743)A

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*HERL has a staff of about 300, over 100 of whom hold doctoral level degrees. The Laboratory has a diverse program which includes **in vitro**, animal, human, clinical, and epidemiological research designed to address issues related to the health effects of air and water chemical and biological pollutants.*

The Human Studies Division (HSD) is a multi-disciplinary program organized into two branches: Clinical Research and Epidemiology with a combined EPA staff of 35, with approximately 75 on-site contractor personnel. The Division is a focal point for planning, conducting, coordinating, supporting and evaluating a program of clinical and epidemiological investigations to improve the understanding of human health risks associated with environmental pollution. The program includes both intramural investigations and extramural arrangements with universities, industry, private research institutions and other government agencies. The primary responsibilities of the Director are to direct and manage the program. This includes developing the program to meet the evolving needs of EPA, presenting the program, developing and managing budgets, supervising the staff, directing the progress of the program and ensuring the scientific quality and productivity of the program.

The preferred candidate for this position would possess the following qualifications:

1. Advanced degree in a biological or medical discipline or epidemiology with at least six years of professional experience in any of these areas. Applicable graduate coursework may be substituted for a portion of the preferred experience.

2. Experience as a supervisor of a scientific/professional staff.

3. Recognized scientific expertise as evidenced by multiple publications in peer-reviewed literature and invitations to participate in national and international meetings/panels.

4. Proven ability to provide leadership in directing the development and progress of a creative and effective multidisciplinary human research program.

5. U.S. citizenship is required.

*Forward curriculum vitae and names of three references prior to May 1 (previously April 17) 1989 to: **Dr. Lawrence W. Reiter, c/o Terri Burrell, HRMD (MD-29), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.***

(NW3408)A

UNIVERSITY OF
essex

OXIDATIVE DEGRADATION OF GLYCATED PROTEINS

Department of Chemistry and Biological Chemistry

Applications are invited for the position of Senior Research Officer to work on the **oxidative degradation of glycated proteins** with Drs Paul Thornalley and Michael Wilson. The formation and oxidative degradation of glycated proteins to advanced glycosylation end-products (AGE compounds) has been implicated in the development of diabetic complications. The project involves studying the kinetics and mechanism of oxidative degradation of model glycated aminoacids, glycated proteins, and finally a search for AGE compounds in plasma from diabetic patients. Applicants should have a PhD or shortly expect to gain one in chemistry, biochemistry or a related subject, and ideally have interests in the biochemistry of hyperglycaemia, especially non-enzymatic protein glycation.

The successful applicant can expect to join a lively and enthusiastic group and enjoy working in a multi-disciplinary life sciences laboratory. The post is funded by the Medical Research Council for three years with salary on the research assistant Grade IA scale (£9,865 - £15,720).

Informal enquiries may be made to Paul Thornalley (Tel. 0206 873010 direct dial) and further particulars can be obtained from the Personnel Office (Tel. 0206 873433).

Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (R/851/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ by 31st March 1989.

(8702)A

EXECUTIVE DIRECTOR

The International Association for Dental Research (IADR) and the American Association for Dental Research (AADR) are seeking an Executive Director, effective July 1, 1990.

The Associations represent the worldwide dental research community with more than 8,000 dental scientists and have joint headquarters in Washington, D.C. USA. The Executive Director is responsible for coordinating the fiscal, administrative, publishing, and conference activities of the two Associations, with a staff of ten. The Executive Director is also responsible for initiating and promoting the interests of dental research worldwide.

Candidates should be qualified to conduct the affairs of a major scientific society. In addition to leadership ability, this involves extensive interaction with members, with other Associations, and with U.S. and other national and international agencies.

Applications and nominations including resume should be received by August 1, 1989, although the search will remain open until the position is filled.

The IADR/AADR is an equal opportunity employer.

Seven copies of the application, and any inquiry or nomination, should be sent to:

Dr. Gordon Rovelstad, Chairman
Search Committee for Executive Director of IADR/AADR
1111 14th Street, N.W., Suite 1000
Washington, DC 20005
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2) Lecturer in Environmental Economics

Applicants should have research interests in the area of the relevant post. Preference will be given to those at an early stage of their careers. The salary will be at an appropriate point on the Lecturer Grade A scale (£9,260-£14,500 per annum, under review).

Applications (three copies) which should include a full curriculum vitae with exact date of birth, together with the names and address of three persons to whom reference may be made, should be lodged with the **Administrative Secretary, University of East Anglia, Norwich, NR4 7TJ (telephone 0603 592734)** from whom further particulars may be obtained, not later than 29 March 1989.

UEA is an equal opportunities employer

(8711)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF EARTH SCIENCES

RESEARCH ASSISTANT/FELLOW

Applications are invited for the above post available from 1 May 1989 for a fixed period of two years for work on geophysical research involving the use of data acquisition and processing techniques.

A good honours degree in Geophysics or Physics and further qualifications/experience in programming, especially in FORTRAN an advantage.

Salary for Predoctoral Research Assistant on R/A Grade IB (£8,675-£11,680) or, for Postdoctoral Research Fellow, on R/A Grade IA (£9,865-£15,720) according to qualifications and relevant experience. Candidates for Research Fellow should have a PhD or equivalent experience.

Informal enquiries may be made to Dr K A Whaler (tel (0532) 335216).

Application forms and further particulars may be obtained from, and completed applications forwarded to the **Registrar, The University, Leeds LS2 9JT, (tel. (0532) 333963 — direct line)**, quoting reference no. 49/37. Closing date for applications — 10 April 1989. (8719)A

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Natural Sciences and Engineering Research Council of Canada

RÉPERTOIRE DES CANADIENS ÉTUDIANT À L'ÉTRANGER

Le Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG) a récemment mis sur pied un répertoire des Canadiens qui poursuivent des études supérieures ou effectuent un stage postdoctoral en sciences naturelles et en génie dans des universités ou des établissements étrangers. Le but de ce répertoire est de faciliter leur retour au Canada en offrant un moyen de communication avec d'éventuels employeurs canadiens.

Admissibilité : les citoyens canadiens et les résidents permanents du Canada qui poursuivent des études supérieures ou qui effectuent un stage postdoctoral dans une université ou un organisme à l'extérieur du Canada dans le domaine des sciences naturelles ou du génie peuvent être inscrits au répertoire.

Fonctionnement : conjointement avec le répertoire, le CRSNG tiendra un registre d'employeurs canadiens éventuels dans les secteurs universitaire, industriel et gouvernemental. Sur demande, un employeur recevra une liste des Canadiens inscrits possédant les compétences spécifiques qu'il aura pré-déterminées.

Le CRSNG établira le contact initial entre les étudiants et les employeurs éventuels; ces derniers seront responsables de toutes communications subséquentes. Ce service est offert gratuitement aux étudiants inscrits et aux employeurs éventuels.

Inscription : les étudiants et boursiers canadiens qui désirent obtenir de plus amples renseignements ou des formules d'inscription doivent communiquer avec :

L'Administratrice du Répertoire des Canadiens étudiant à l'étranger
Conseil de recherches en sciences naturelles et en génie du Canada
200, rue Kent
Ottawa, Canada
K1A 1H5
Téléphone : (613) 995-6060

Canada

(W5974)A

GLASGOW UNIVERSITY
Chemistry Department

POSTDOCTORAL RESEARCH ASSISTANT

The post, funded by SERC, involves novel studies of stereochemistry and magnetic structure in a range of organic, inorganic and biological molecules using a multichannel Raman optical activity instrument. Applicants should have a background in chemical physics.

The appointment will be made on an annual basis, renewable for up to three years, starting on 1st August 1989 or on an agreed date up to six months thereafter. Salary is within the 1A Range for Research and Analogous Staff (£10,460-£11,680, plus USS).

Applicants should write directly to **Professor L. D. Barron (Chemistry Department, the University, Glasgow G12 8QQ)** enclosing a C.V. and the names and addresses of two academic referees. (8748)A

UNIVERSITY OF BIRMINGHAM

LECTURER

in the Medical Science School attached to the Department of Immunology

Applications are invited from established research scientists in the field of molecular immunology with a particular interest in the regulation of gene expression during the development, differentiation and activation of leucocytes. The holder of this post will be expected to form his or her own research group which will be largely self funded by external grants. The successful candidate will be chosen for their potential for interaction with other research groups working on leucocyte physiology and pathology in the University. These include Professor J J T Owen, T cell development; Professor I C M MacLennan, B cell physiology; Professor A B Rickinson, viral oncogenesis of B cells; Dr G Brown, regulation of granulocyte and macrophage development and Professor R Michell, intracellular transduction pathways in leucocytes. An important level of cross collaboration already exists between these groups which are respectively located in the Departments of Anatomy, Immunology, Cancer Studies, Immunology and Biochemistry. The appointee will be expected to participate in undergraduate and postgraduate teaching in the field of immunology and molecular genetics.

Salary in the range £9,260-19,310 p.a. plus superannuation.

Further particulars and application forms may be obtained from the **Senior Assistant Registrar, University of Birmingham Medical School, Vincent Drive B15 2TJ (Tel: 021-414-4049)** to whom application forms including CV and the name of three referees should be sent by not later than 28th April 1989.

AN EQUAL OPPORTUNITY EMPLOYER (8764)A

HATFIELD POLYTECHNIC

SCHOOL OF NATURAL SCIENCES

L/SL PLANT GENETICS
(REF. 892)

L/SL LANDSCAPE ARCHITECT/PLANNER
(REF. 893)
SALARY: LECTURER £8,814-£15,702/SENIOR LECTURER £15,702-£18,882

Following the establishment in 1988 of a degree in horticulture in collaboration with Writtle College, these two full-time appointments are available from 1 September 1989 in the Polytechnic.

The plant Geneticist will be based in the Division of Biological Sciences and will be expected to teach plant breeding methods, cytogenetics and basic biochemistry to students of horticulture and applied biology. Applicants should have either postgraduate or postdoctoral experience, preferably in commercial horticulture and will be expected to pursue relevant research and participate in short courses and consultancy..

Informal enquiries may be directed to Dr I Simkins (07072 79552).

The Landscape Architect/Planner will be based in the Division of Environmental and Earth Sciences and will be expected to teach landscape studies (historical development, policy, management and design) and environmental planning (development control and the planning process). Applicants will be expected to actively pursue research in a relevant area and to participate in course design and administration, short courses and consultancy.

Informal enquiries may be directed to Dr K Bardon (07072) 79516.

L/SL MICROBIAL MOLECULAR GENETICS
(REF. 894)
SALARY: LECTURER £8,814-£15,702/SENIOR LECTURER £15,702-£18,882

Applications are invited from postdoctorates in microbial molecular genetics. The successful candidate will be expected to contribute to teaching and development of genetics and molecular biology in undergraduate, postgraduate and short courses and to carry out research in some aspect of molecular biology. Experience with yeast systems would be an advantage but not essential. Informal telephone enquiries may be addressed to Mr P F Stanbury (07072) 79550. The appointment is available immediately.

Closing date for all the above posts 7 April 1989.

Further details and application forms may be obtained from the Personnel Department, Hatfield Polytechnic, College Lane, Hatfield, Herts AL10 9AB or telephone Hatfield (07072) 79802.

Please quote appropriate reference number when applying.

(8767)A

POSTDOCTORAL OPPORTUNITIES

Howard Hughes Medical Institute, a leading medical research organization, has postdoctoral positions available to study the structure-function relationships of blood clotting proteins or cellular regulation of receptor expression.

You will work in a new facility with monoclonal, tissue culture and structural biology core facilities. Experience in molecular or cell biology, or protein or physical chemistry is preferred.

To apply, send C.V. and two recommendations to: **Charles T. Esmon, PhD, Howard Hughes Medical Institute, c/o Oklahoma Medical Research Foundation, 825 NE 13, Oklahoma City, OK 73104. EOE.** (NW3441)A

Immediate position for
POSTDOCTORAL FELLOW
(M.D. or Ph.D.)
interested in gene transfer and gene therapy. Ongoing project in molecular and cellular biology transferring human globin genes in retroviral vectors into mice. Previous experience in molecular biology preferred.

Send résumé to: **Dr. Arthur Bank, Columbia University, HHSC 1602, 701 West 168th Street, New York, NY 10032.** (NW3450)A

UNIVERSITY OF EDINBURGH
FACULTY OF MEDICI
DEPARTMENT OF ANATOMY

TWO LECTURESHI (NON-CLINICAL) I HUMAN ANATOM

Applications are invited for two tureships in the Department of A omy for which the University sought funding under the UGC's academic appointments sch with effect from October 1989. U the terms of this scheme, whic intended to provide perma appointments for young staff, pr ence will be given to candidates *early stage in their careers.* The ir iduals appointed should ideall clinically qualified with some ex ience in the teaching of Topogra cal Anatomy. Candidates should ferably also be familiar with the te ing of Histology, Neuroanatomy Embryology, and be actively invol in anatomically oriented/clinic relevant research. Medically or sc tifically qualified candidates witho PhD or equivalent will be conside as staff in post are encouragec study for a higher degree or diplor

Salary will be at an appropriate p on the Lecturer scale AI2A (prese maximum £14,500 per annum) v superannuation under the Unive ties Superannuation Scheme.

Further particulars may be obtai from the Personnel Office, Univer of Edinburgh, 63 South Bridge, Ec burgh EHL 1LS.

Applications by letter (6 copies), cluding c.v. and names and addres of two referees should be sent **Professor M.H. Kaufman, Univers Medical School, Teviot Place, Ed burgh EH8 9AG,** not later than 1 April 1989. Please quote referer NAAS2002. (8721)A

POSTDOCTORAL/RESEARCH ASSOCIATES

THEORETICAL/ COMPUTATIONAL CHEMISTR

EXPERIMENTAL FEMTOSECON SPECTROSCOPY

Molecular dynamics of chemica reactions in solution, includin enzyme and other catalyze reactions. Comparison amon molecular dynamics calcula tions, analytical theories an experimental measurements.

Prof. Kent R. Wilson, Department of Chemistry B-039 University of California, San Diego, La Jolla CA 92093.

Affirmative Action/Equal Oppor tunity Employer. (NW3189)A

Where is the university graduate (m/f) who takes the responsibility for the research strategy of the future as Head of Section, Pharmacology?

Duphar B.V. is a medium-sized internationally orientated company with a range of ethical pharmaceuticals originating from its own research and development.

Duphar reinvests a large proportion of its turnover in R & D. Major areas of research are in the central nervous, vascular and circulatory systems.

The R & D facility is situated in pleasant surroundings, sixteen kilometers from Amsterdam.

Duphar's Pharmacology Department (about 75 staff) is housed in well-equiped new laboratories. Due to an internal promotion this department has a vacancy for a head of vascular and circulatory pharmacology.

This important managerial position offers an excellent opportunity to participate in steering the future research strategy in the vascular and circulatory field. You will manage a multidisciplinary team of seven PhD's, about 25 competent technicians and 3 research assistants. The research field includes atherosclerosis, ischemia, fibrinolysis and migraine.

The successful candidate for this position will have a PhD and broad knowledge of vascular and circulatory pharmacology. Through a proven record of publications he or she must demonstrate his/her ability to think creatively in a research environment.

The candidate should be able to interact with our chemists as well as our clinicians. The candidate must have significant management experience and be able to integrate the multidisciplinary team. Experience within the pharmaceutical industry is essential for this important position.

For further information please call Dr A. de Jonge, Director of Pharmacology, 312940-79633. Applications including C.V. should be sent to Mr J.W. Rengelink, Personnel Department, Duphar B.V., P.O. Box 2, 1380 AA Weesp, The Netherlands, mentioning vacancy number 4251.

duphar

(W5971)A

PENNSTATE

The Pennsylvania State University
College of Medicine

NEUROSCIENCE RESEARCH IN ANESTHESIA

The Department of Anesthesia of The Pennsylvania State University College of Medicine and the University Hospital of The Milton S. Hershey Medical Center is inviting applications for a research faculty position to begin in the Summer or Fall of 1989. The Department presently has four areas of concentration in research related to physiology and pharmacology of the anesthestic state: including cardiovascular, pulmonary, neurophysiological and neuropharmacological areas. We are seeking candidates with a Ph.D. or M.D. qualification with substantial post doctoral experience in one or more of the following areas: mechanisms of drug action, neurotransmitters, membrane receptors, signal transduction, ion-channels, and mechanisms of consciousness. We are particularly interested in candidates who are able to relate their research to the questions of mechanisms of pain and anesthesia. Significant research accomplishment and demonstrated ability to obtain independent funding are essential. Excellent opportunities exist for research collaboration and a joint appointment in a basic science department and participation in the Neuroscience Program.

Applicants should submit, prior to May 1, 1989, curiculum vitae, reprints, statement of current and planned research interests, and names of at least three references to:

Julien F. Biebuyck, M.B., D.Phil.
Eric A. Walker, Professor and Chairman
Department of Anesthesia
THE PENNSYLVANIA STATE UNIVERSITY
COLLEGE OF MEDICINE
THE MILTON S. HERSHEY MEDICAL CENTER
P.O. Box 850
Hershey, PA 17033

Equal Opportunity/Affirmative Action Employer (NW3429)A

FACULTY POSITION IN MOLECULAR DEVELOPMENTAL GENETICS

RESEARCH ASSISTANT PROFESSOR

Center for Inherited Diseases
University of Washington

The University of Washington School of Medicine seeks applications for a Research Assistant Professor position in the Division of Medical Genetics and the Center for Inherited Diseases. We are particularly interested in identifying molecular biologists who wish to pursue research initiatives in molecular developmental genetics. The successful applicant will participate as a coinvestigator in a research program focused on the analysis of molecular control of globin gene switching and will also be expected to develop an independent research program on some aspect of molecular developmental genetics.

Funding is available for three years. Candidates should forward a curriculum vitae, three letters of reference, and a statement of research interests to **Dr. George Stamatoyannopoulos, Division of Medical Genetics, RG-25, University of Washington, Seattle, WA 98195.** Deadline for applications is May 15, 1989. *The University of Washington is an Equal Opportunity/Affirmative Action Employer.*

(NW3433)A

RESEARCH SCIENTISTS MAMMALIAN CELL GENE EXPRESSION

Amgen, an industry leader in applied biotechnology, has an immediate need for 2 Research Scientists to contribute to our research programs in mammalian cell gene expression.

RESEARCH SCIENTIST CELLULAR PHYSIOLOGY

Dept. #347

This position requires a Ph.D. in mammalian cellular physiology and/or somatic cell genetics as well as a minimum of 2 years' postdoctoral experience in growth regulation and differentiation of animal cells. Familiarity with culturing various primary and established cell lines as well as experience in cloning and sorting cells and selecting metabolic and drug resistant mutants are essential. Some understanding and experience in cell culture scale-up are desirable.

RESEARCH SCIENTIST GENE REGULATION

Dept. #348

Qualified candidates for this position will have a Ph.D. and a minimum of 2 years' postdoctoral experience in eukaryotic post-transcriptional gene regulation, including protein processing and secretion. Familiarity with glycosylation pathways and signal transport at the molecular level is essential.

Amgen offers an exciting and challenging research environment as well as an excellent compensation and benefits package including equity offerings. Qualified applicants should send a c.v. including references, in confidence, to:

AMGEN, INC.
Recruitment, Specify Dept. #
1900 Oak Terrace Lane
Thousand Oaks
CA 91320 (NW3432)A

Equal Opportunity Employer

AMGEN

NATIONAL INSTITUTES OF HEALTH
IMMUNOLOGIST/ CELL BIOLOGIST

Experience in immunology, cell biology and molecular biology to study the role of human $CD5^+$ B lymphocytes in the normal and autoimmune repertoire and to make human monoclonal antibodies. The candidate should have at least two years postdoctoral experience. The initial appointment will be for two years, with renewals on a yearly basis. Starting salary range, depending on experience, is $30,000 to $50,000 per year. The position is available immediately. Send Curriculum Vitae and names of references to **Dr. Lois Salzman, Deputy Director Intramural Research Program, National Institute of Dental Research, National Institutes of Health, 9000 Rockville Pike, Building 30, Room 121, Bethesda, Maryland 20892.** NIH is an Equal Opportunity Employer.

(NW3436)A

EG&G BIOMOLECULAR™

OPPORTUNITY IN AUTOMATED DNA SEQUENCING INSTRUMENTATION

EG&G BIOMOLECULAR, a leading US supplier of automated DNA Sequencing Instrumentation is expanding its European activities.

EG&G BIOMOLECULAR is a division of **EG&G**, a Boston-based FORTUNE 500 company with a 40-year tradition of leadership in science and technology.

To support our distribution organizations in Europe we are looking for the

EUROPEAN APPLICATIONS AND PRODUCT SPECIALIST DNA SEQUENCING

based in our European Distribution Headquarters in Munich, West Germany.

You will provide application advice to customers and technical support to our sales engineers throughout Europe, develop and conduct training programmes for our automated DNA sequencer, and attend seminars and exhibitions. You will work closely with customers and scientific advisors to identify new product opportunities and monitor market trends. The close contact with our sales organizations and the cooperation with our marketing department in the US will require extensive travel within Europe and the USA.

- ★ *Do you enjoy working in a team and a people-oriented environment? Is your participation in starting this new busines exciting you?*
- ★ *We offer an attractive salary augmented by a performance related bonus scheme as well as the benefit of a company car.*
- ★ *You will have a degree in advanced life science, experiencing in DNA sequencing, and working experience in a molecular biology laboratory.*

Become part of **EG&G**. Send your resume and photograph to

EG&G GmbH
Hohenlindener Str. 12
D-8000 Muenchen 80
Phone: (089) 92692-111
(W5980)A

BTG
British Technology Group

BTG is the largest and most successful technology transfer organisation in the world – it promotes the development of new technology into commercial products.

BTG's Pharmaceuticals Division has a major involvement in the development and licensing of innovative pharmaceuticals and biologicals for human and veterinary use and we are now seeking two Executives to join an existing professional multi-disciplinary team.

Cancer Specialist

Three years ago BTG launched a major initiative to encourage research into the development of new agents for the diagnosis and treatment of cancer. We now require a scientist with broad based experience in the field of cancer to manage the existing programmes and to initiate new lines of product orientated research.

Pharmaceutical Scientist

For the second post we are seeking a person with a research background and knowledge of drug discovery and development. The successful candidate will be expected to identify and evaluate new therapeutic opportunities in a broad area of medical research.

FOR BOTH POSTS responsibilities will include liaising with research laboratories primarily in the UK academic sector, and setting up and managing BTG-funded development projects. The overall aim is to expand BTG's portfolio of inventions for patenting and licensing to industry worldwide. Responsibilities will also include selecting licensees for particular inventions. The posts are based in London but considerable travel is involved.

Applicants aged 30+ should preferably possess a higher degree, but the subject is of less importance than post-graduate experience. A period in pharmaceuticals is desirable for the first post and essential for the second, ideally involving drug development.

Major company benefits include relocation assistance if appropriate.

For an informal discussion telephone 01-403 6666 Ext. 2525. Otherwise contact the Personnel Manager for an application form or send CV, including salary, to her at: British Technology Group, 101 Newington Causeway, London SE1 6BU. Telephone 01-403 6666.

(8766)A

DEPARTMENT OF GEOLOGICAL SCIENCES

Lecturer in Geology

– PLYMOUTH CAMPUS

Salary: Within the range £8,481 to £15,369 (starting point dependent on experience and qualifications) – normally with further advancement to £18,549 per annum.

Applications are invited from suitably qualified geologists to join a strong and very active department. Candidates will be expected to teach on the BSc Applied Geology degree and take a leading role in the geological components of the BSc Environmental Science degree. The successful candidate is likely to have a substantial research record and will have either worked in industry or have experience of professional practice. To balance our present expertise, applications are particularly sought from the fields of hydrogeology, industrial minerals, micropalaeontology, remote sensing or mathematical geology.

Application forms, to be returned by Friday 31 March 1989, and further details are available from the Personnel Department at the address below, or by telephoning (0752) 264639.

Plymouth Polytechnic (8710)A

Drake Circus, Plymouth, Devon PL4 8AA

AN EQUAL OPPORTUNITIES EMPLOYER

UNIVERSITY OF BRISTOL
DEPARTMENT OF PATHOLOGY

Applications are invited for the post of **RESEARCH ASSISTANT** for one year in the first instance. The project concerned is funded by the Cancer Research Campaign to carry out DNA analysis on extracts of Sternberg-Reed cells from Hodgkin's lymphoma.

It is expected that the applicant will have some experience of nuleic acid hybridisation techniques and/or cell biology. The required techniques are currently in use in this Department of Pathology, so training is available.

Initial salary in the range £8675-£9865 (Research Assistant Grade 1B) according to age, qualifications and experience.

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to **Personnel Office, Senate House, Bristol BS8 1TH.** Please quote reference A261.

Informal enquiries about the post may be made by telephone to Dr. N Rooney (0272-230000 – Ext. 2869). (8717)A

DEPARTMENT OF TRADE AND INDUSTRY

Laboratory of the Government Chemist
Teddington, Middlesex

Research Worker

We have recently embarked upon a research programme in analytical molecular biology (AMB) aimed at the development of practical techniques for the detection and identification of specific (and foreign) DNA sequences in the presence of a large excess of genomic DNA. Many applications to analytical science are envisaged. The work is being performed in collaboration with King's College, London.

We are looking for a research worker to lead the small AMB team and to be responsible for the practical management of the programme and liaison with analytical groups in the LGC and with workers at King's. Specific objectives will include the management and training of staff of the LGC, development of contact with workers in complementary areas of science, and the presentation of results of the programme at symposia and in the scientific literature.

You should have a 1st or 2nd class honours degree in biochemistry, molecular genetics or molecular biology with at least 2 years' post-graduate experience. A PhD and experience of recombinant DNA technology and DNA cloning and sequencing are preferable.

Starting salary will be in the range £11,230-£14,730 (including £725 Outer London Weighting) with further increments, depending on performance, up to £17,100.

RELOCATION ASSISTANCE UP TO £5000 MAY BE AVAILABLE.

For further details and an application form (to be returned by 31 March 1989) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours). **Please quote ref: SA/15/MB.**

The Civil Service is an equal opportunity employer

Scientific
CIVIL SERVICE

(8756)A

MAX-PLANCK-INSTITUTE OF BIOCHEMISTRY

Staff Scientists (BATIIa)
Postdoctoral Fellows
Research Technicians

Several positions are available immediately in the newly established Department of Molecular Biology. Successful applicants will have extensive experience in one or several of the following research disciplines: molecular biology, cell biology, cell physiology, and protein chemistry and purification. The research program of the new Max-Planck division will focus on structure/function analysis of cell surface receptors, the molecular basis of receptor-mediated signal transduction, and the involvement of growth factors and their receptors in human cancer.

Please send applications including CV and names of two referees to **Dr. A. Ullrich, Max-Planck-Institut fur Biochemie, D-8033 Martinsried bei Munchen, F.R.G.**

(W5966)A

CIBA-GEIGY Animal Health is seeking qualified individuals to fill open positions within its BIOVET Unit, a young and dynamic R & D group established in 1985 to pursue product opportunities in animal health care based on biotechnology-derived materials. Specifically, we look for a

R & D Scientist

with a DVM degree plus a minimum of 2-3 years of post-graduate training (such as residency, industrial experience or a PhD) in the areas of Internal Clinical Medicine and/or Pathology. We also look for a

Postdoctoral Fellow

with a DVM degree plus further training in Immunology or Microbiology.

Candidates for both positions must have a thorough knowledge of the livestock industry in North America or Europe and a minimum of one year experience in general veterinary practice. Expertise in data management and statistics is desirable.

The prospective members of the team will be working in our R & D center situated in the French-speaking area of Switzerland. They will have the excellent opportunity to work in a highly interactive environment and to contribute actively to the solution of practical problems in animal health care.

Please send your application with documented history under code "Nature 238" to Mr. H. Schmid, CIBA-GEIGY Ltd., Personnel Department, P.O. Box, CH-4002 Basle.

(W5975)A

CIBA–GEIGY

ASTROPHYSICS DEPARTMENT, SACLAY, FRANCE

Tenure-track Position in Extragalactic Astrophysics

The Astrophysics Department of the Center for Nuclear Studies, Saclay, France, invites applications for a tenure-track position in the general area of extragalactic astrophysics. The Department is part of the General Physics Division of the Institute of Fundamental Research of the Commissariat à l'Energie Atomique, the French Atomic Energy Commission. Current, but not exclusive, interests of the Department are on galaxy evolution, clusters of galaxies, large-scale structures in the universe, along with star formation regions, stellar evolution, nucleosynthesis and compact binaries. The Department is heavily involved in the design and manufacturing of instruments for the main European space observatories ISO and XMM, as well as the γ-ray imager "Sigma", soon to be launched aboard the Soviet satellite Granat.

The successful applicant is a senior scientist, who will interact with the various groups of the Department. He is expected to strengthen theoretical and observational programs, in connection with the above space experiments. The position is for six months to one year, starting in fall 1989 or winter 1990, with a high probability of tenure at the end of this term. There is no restriction on nationality. Salary will be commensurate with experience, with a net minimum of about 200 000 FF per year. Applicants should send a statement of research interests and projects, vita, publication list, and arrange to have three letters of recommendation sent to **Dr. Catherine Cesarsky, Head,** at the address below.

The deadline is May 31, 1989.

Postal address: Service d'Astrophysique, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex,France. Fax: +33 1 69 08 65 77. E-mail (Span): 32779: NOELLE.

(W5963)A

POSTDOCTORAL PHARMACOLOGIST

Pfizer is a world leader in the field of therapeutic drug research and development. In order to maintain the momentum and productivity of our research programmes we are seeking to recruit an enthusiastic young Pharmacologist with broad experience of *in vivo* techniques to our General Biology Group.

The role of the General Biology Section is to evaluate the effects of novel chemical entities on physiological systems to follow up unexpected observations and to develop new models of pathophysiological disease processes. This involves *in vitro* and *in vivo* evaluation using classical and biochemical, immunological and pharmacological techniques. To cover this spectrum of activities, the team consists of approximately 10 individuals and a vacancy has arisen for a senior pharmacologist/physiologist.

If you enjoy the challenges of discovery research and can transmit your enthusiasm to others then this is an ideal opportunity to work in modern, well-equipped laboratories, supported by the technical and financial resources of one of the world's foremost and highly respected pharmaceutical companies.

This is a particularly exciting time to join Pfizer as we have a number of new therapeutic agents being launched on world markets and others reaching the final stages of their development. We also have a multi-million pound expansion of our research facilities under construction, demonstrating our long-term commitment to research here at Sandwich and enabling our scientists to look forward with confidence to the 1990's and beyond.

If you join us, you will be paid a good salary, be eligible for an annual performance bonus of up to 20% of salary, participate in our excellent employment benefit package of pension, share ownership and private medical insurance schemes and be able to live in one of East Kent's charming towns or villages. Naturally, we will pay your removal expenses.

If this advertisement attracts your interest, please write in confidence, giving full details of career to-date to Mr D O'Callaghan, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ. Tel: (0304) 616181.

Pfizer

(8782)A

THE UNIVERSITY OF LEEDS
SCHOOL OF MATERIALS
(DIVISION OF CERAMICS)

PREDOCTORAL RESEARCH ASSISTANT/POSTDOCTORAL RESEARCH FELLOW

A vacancy exist in the area of Silicon nitride — titanium nitride particulate composites to join a small team working in related aspects of the programme.

The post is available immediately for a fixed term of up to one year; there is a possibility of appointment for a further, shorter, period becoming available.

Candidates for Research Assistant should have a good honours degree in an appropriate discipline, and for Research Fellow a PhD or equivalent industrial experience.

Salary for Research Assistant on R/A IB (£8675-£11680), and for Research Fellow on R/A IA (£9865-£12760), according to qualifications and relevant experience.

Informal enquiries to Dr. F. L. Ridley (tel. 0532 332541) fax: 0532 42253.

Application forms and further particulars may be obtained from and completed applications sent to the **Registrar, the University, Leeds LS2 9TJ, (tel. 0532 333965)** quoting reference no. 62/29. *Closing date for applications 6 April 1989. (8731)A

Sandoz Institute for Medical Research

Senior Scientist

Neuropharmacology

Applications are invited for the post of Senior Scientist in the Laboratory of Neuropharmacology at the Sandoz Institute. This laboratory uses a variety of methods, including electrophysiology, to examine drug actions on the peripheral and central nervous system. These studies are part of interdisciplinary programmes concerned with the development of novel analgesics and with basic research in neurobiology.

The Sandoz Institute occupies modern laboratories within the premises of University College, London. It comprises some 60 permanent staff as well as post doctoral fellows and research students. A range of biological expertise and interests are represented and fostered.

Candidates for the Senior Scientist position should have post doctoral experience. A knowledge of pharmacology and expertise in electrophysiology would be desirable.

Starting salary would be in the range of £19,500-£24,000 – depending on age and experience. Those interested are invited to apply or write for more information. Applications should include a curriculum vitae and the names of two referees. Send to

Ms M-C. Stuart, Sandoz
Institute for Medical Research,
5 Gower Place, London WC1E 6BN.
Closing date: 30 April 1989.

SANDOZ

(8752)A

An exciting opportunity in Molecular Biology

c.£13,500
Beckenham, Kent

- **The Wellcome Foundation Ltd.**
- **Major International Pharmaceutical Group**
- **Research Centre at Beckenham**
- **Annual Turnover >£1,250m**
- **An Equal Opportunity Employer**

The Wellcome Foundation is internationally renowned for its innovative research and the development of new medical products, many of which represent significant advances in human health care. These developments are dependent upon maintaining an extremely high standard of research activities within the Company.

The recently established Department of Molecular Sciences currently has an exciting opening for a talented and highly motivated young scientist to join an existing WHO-funded research programme aimed at developing new chemotherapeutic agents for the treatment Onchocerciasis (river blindness). We are seeking a molecular biologist to work alongside established scientists in a multi-disciplinary team. This will be a one year appointment with good prospects of renewal.

Candidates should possess a Ph.D. in molecular biology with an interest in applying their skills to challenging problems in parasitology.

Salaries will be in excess of £13,500, dependent on age and qualifications.

The company offers excellent benefits including 5 weeks' holiday, pension scheme, subsidised restaurant and first-class sports and social facilities on site.

Please write with full career details to Miss S. J. McKinlay, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, quoting reference number 91/69.

(8780)A

Wellcome

UNIVERSITY OF OXFORD

NEUROSCIENCES GROUP
Institute of Molecular Medicine

SENIOR RESEARCH FELLOW

Grade IIX Salary £15,105-£20,615

Applications are invited for a senior post-doctoral scientist to take a prime role within a group investigating autoimmune disorders of ion channel function at the neuromuscular junction. An essential requirement for the successful candidate would be an extensive background in ion channel biochemistry together with considerable experience in immunochemistry and recombinant DNA techniques. Starting date October 1st, 1989.

Letters of application, together with cv and the names and addresses of two referees should be sent to **Professor J. Newsom-Davis, Neurosciences Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU.**

POST-DOCTORAL RESEARCH ASSISTANT

Grade IA Salary £9,865-£15,720

A biochemist is required for a three-year MRC funded project to investigate neuronal cytoskeletal abnormalities in neurological disease. Experience of protein biochemistry would be an advantage. Starting date negotiable.

Further particulars of the appointment may be obtained from **Dr Michael Donaghy, University Department of Clinical Neurology, Radcliffe Infirmary, Oxford OX2 6HE**, to whom applications, including a cv and the names of two referees, should be sent.

Closing date for applications for both posts will be April 7th.

(8726)A

The University is an Equal Opportunity Employer

RESEARCH ASSOCIATE

Research Associate position available starting July 1989 at Bascom Palmer Eye Institute, Department of Ophthalmology, University of Miami School of Medicine, Miami, Florida, to study molecular aspects of human genetic ocular diseases. Recombinant DNA techniques are primarily used to study retinitis pigmentosa gyrate atrophy and retinoblastoma.

Applicant should have Ph.D./M.D. Experience in molecular biology preferred but not required.

Send curriculum vitae and names, addresses and telephone numbers of three references to: **Dr. George Inana, National Institutes of Health, Building 6, Room 232 Bethesda, MD 20892.**

University of Miami is an Equal Opportunity/Affirmative Action Employer.

(NW3461)A

ASSISTANT OR ASSOCIATE PROFESSOR OF ANATOMY

Applications are invited for tenure-track positions at the assistant or associate professor level. Applicants at the assistant professor level should have a Ph.D., M.D., or equivalent, postdoctoral experience, an active research program with a high potential for attracting external support, and promise of ability as a teacher. Applicants at the associate professor level should have an ongoing, externally funded research program, and established record of scholarship supported by substantial publications, teaching experience, and a record of successful direction of doctoral candidates or fellows. Specific research areas are open, although preference will be given to cellular and molecular biology. Areas of specific teaching expertise are also open, although experience in or ability to teach gross human anatomy is highly desirable. *Women and minorities are encouraged to apply.*

Send curriculum vitae, bibliography, a brief statement of research and teaching experience, plus names of three references to: **Chair, Anatomy Faculty Search Committee, The University of Iowa College of Medicine, Iowa City, IA 52242.**

The University of Iowa is an Equal Opportunity/Affirmative Action Employer.

(NW3462)A

UNIVERSITY OF EDINBURGH

)epartment of Botany

Postdoctoral Research Associate

postdoctoral research asso-ate is required to investigate e basis of hyphal guidance id infection structure differ-itiaton in the bean rust ngus. Fluorescence ratio aging will be used to deter-ine the roles of intracellular alcium and pH in these pro-sses. This A.F.R.C.-funded oject will be supervised by rs. N. D. Read and A. J. rewavas.

he post is tenable for three ears. Applicants should send curriculum vitae with the ames of two referees to **Dr. N. . Read, Dept. of Botany, niversity of Edinburgh, ing's Buildings, Mayfield oad, Edinburgh EH9 3JH.** lease quote reference no. 648. (8750)A

UNIVERSITY OF MANCHESTER

RESEARCH ASSISTANT/ASSOCIATE

Applications are invited for the above post, in the Department of Cell and Structural Biology, from postdoctoral candidates or graduates to work on a project concerned with the interplay between matrix macromolecules and soluble growth factors in the regulation of fibroblast function during wound healing. Extensive use will be made of cell culture techniques. Excellent facilities are available for all cell and molecular biological techniques. Initial funding is available for three years on salary range £9,865-£11,680 p.a. (postdoctoral) or £8,675-£10,460 p.a. (graduate).

Please forward complete CV and names of at least two academic referees to: **Dr Seth L. Schor, Department of Cell and Structural Biology, the University, Manchester M13 9PL.** The University is an equal opportunities employer. (8718)A

KEEPER OF MINERALOGY

LONDON

Mineralogy is one of five major scientific departments within the British Museum (Natural History). As well as housing one of the world's leading collections of minerals, meteorites and rocks, the Department operates a superbly-equipped research unit featuring the most advanced analytical systems, including an electron microscope and an infra-red spectrometer. The Museum now wishes to appoint a scientist of considerable professional standing to lead this prestigious Department.

The Keeper has wide-ranging curatorial, scientific and managerial responsibilities, and enjoys great influence within the scientific community. As well as being responsible for the care and development of the collections, the new Keeper will direct the work of a 30-strong research team which includes acknowledged experts in various aspects of mineralogy. The managerial component of the role embraces everything from financial control to the leadership of all grades of staff.

The successful candidate will be a scientist whose work in mineralogy or related disciplines has already won an international reputation. A knowledge of the care and maintenance of mineral collections and strong administrative abilities are also essential.

Starting salary (from April 1989) is in the range £29,920-£33,350 (including £1750 Inner London Weighting) with further increments depending on performance up to £38,535.

For further details and an application form (to be returned by 14 April 1989), write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours.)

Please quote ref: S/7882.

An equal opportunity employer.

(8769)A

DIRECTOR FOR NEW AIDS RESEARCH CENTER AND LABORATORY

A strong basic scientist experienced in the molecular biology of AIDS is sought to direct the activities of the new Aaron Diamond AIDS Research Laboratory for the City of New York, sponsored and supported jointly by New York University School of Medicine, the Department of Health of New York City, the Public Health Research Institute and The Aaron Diamond Foundation. Governance of the center is by a Board of Directors drawn in part but separate from the sponsoring institutions.

Creation of a strong research environment and the prosecution of molecular virologic and/or immunologic research on AIDS and related diseases should be the Director's primary objectives; the center should serve as a focus for collaborative activities involving scientists and physicians throughout New York City.

Abundant research space that will include BL-3 containment facilities is under construction. Start-up funds have been provided. The position carries a tenured professorship at NYU; salary will depend on qualifications.

Inquiries including curriculum vitae and bibliography should be sent to the Chairman of the Board of Directors:

Dr. Edwin D. Kilbourne, Box 1124, Mount Sinai School of Medicine, 1 Gustave Levy Place, New York, NY 10029.

(The Board is committed to Equal Opportunity/Affirmative Action in employment.) (NW3446)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

LECTURER/SENIOR LECTURER IN NEUROSCIENCES

Applications are invited for an established lectureship or senior lectureship in neurosciences. The post will be held in one of the preclinical departments, according to the research interests of the successful applicant. Preference may be given to applicants with research interests in the central nervous system or in integrative aspects of neurosciences. The level of appointment will depend on qualifications and experience. Informal enquiries may be made to the Chairman of the Department of Physiology, Professor J G Widdicombe (01-672 9944 ext 55390).

Further particulars and an application form from the **Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE** (01-672 9944 ext 56020). Closing date 12 April 1989. Quote reference 38/89. (8744)A

UNITED MEDICAL AND DENTAL SCHOOLS

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RESEARCH ASSISTANT IN MOLECULAR SCIENCE

required for cloning and sequencing of some T cell surface receptors. Graduate of Postdoctoral scientists may apply if they have experience in molecular biology or protein chemistry.

Salary, according to qualifications and experience, in range £8,675 to £14,500 plus £1,650 London Allowance. Appointment is for three years.

For further information, please contact Professor Thomas Lehner or Dr Charles Kelly: 01-407 7600 ext 3960/2965.

Applications, with full curriculum vitae and names of two referees, to **Staffing Office, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH,** quoting Ref: G./IMM/284. (8714)A

TheHospital for Sick Children, a fully accredited, 650-bed paediatric teaching hospital, affiliated with the University of Toronto, seeks a

Pathologist-in-Chief

This is a joint appointment with the Department of Pathology, University of Toronto.

The hospital department has a well-equipped laboratory with facilities for electron microscopy, immunohistochemistry, cytology and histology as well as a medical staff of seven. Applicants should be paediatric pathologists with an established research program, excellent teaching skills and appropriate administrative experience.

Candidates must have a FRCPC or equivalent and be eligible for licensure in the Province of Ontario. In accordance with Canadian Immigration requirements, this announcement is directed to Canadian citizens and permanent residents. Potential candidates should submit their curriculum vitae by May 30, with the names of 4 referees, **quoting file # D021C,** to: Mary Babyn, Pathology Committee, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario Canada M5G 1X8

(NW3457)A

HSC THE HOSPITAL FOR SICK CHILDREN

Research Associate/ Cell Biologist

La Jolla, California

Pharmacia Experimental Medicine is the research unit of Pharmacia Inc., conducting explorative research in microcirculation/inflammation.

We are currently seeking a highly motivated postdoctoral research associate to work on the cellular and molecular aspects of leukocyte/endothelial cell functions. The successful candidate should have an interest in inflammation / ischemia-reperfusion as related to attachment, diapedesis and motility of cells in connective tissue. Biochemical experience is required.

We offer excellent training in microcirculation and beautiful surroundings in La Jolla, California. For consideration please send your resume to **Dr. Karl-E. Arfors, Director of Explorative Research, Pharmacia Experimental Medicine, 11099 North Torrey Pines Road, La Jolla, CA 92037.** (NW3474)A

Pharmacia

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

POST-GRADUATE CHEMIST GRADE 1B

BC/279

A vacancy now exists for a post-graduate organic chemist to synthesize deuterated lipophilic molecules, specifically ubiqunione-10, using published methods. The position is available for nine months in Dr. A. Watts laboratory.

Salary on the scale £8,675-£13,365 depending upon age and experience.

Applications, including a curriculum vitae and the names and addresses of two referees who can comment on practical experience, should be addressed to: **The Administrator.**

An Equal Opportunity Employer. (8727)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD, OX1 3QU.

FACULTY POSITIONS

University of Puerto Rico Medical Sciences Campus

Accredited research and teaching institution with teaching in either Spanish or English will fill the following tenure-track positions in the next two years: **PHYSIOLOGY**: Four positions, rank open, in cell/systems physiology. **BIOCHEMISTRY**: Three positions: i.) Professor and Chair, ii.) Assoc. Professor with research in protein chemistry, iii.) Asst. Professor. **ANATOMY**: One position, rank and research area open, teaching in medical gross anatomy. **MICROBIOLOGY**: Two positions, Asst. Professor level: i.) molecular biologist, ii.) parasitologist with research in immunoparasitology or molecular biology of parasites. **ELECTRON MICROSCOPY**: Director of central EM unit. **NEUROBIOLOGY**: Two positions in biophysics or comparative electrophysiology. Applicants should send a CV, reprints and the names and telephone numbers of three references to: **Dr. Susan Opava-Stitzer, Director, RCMI Program, GPO Box 5067, UPR Medical Sciences Campus, San Juan, PR 00936 U.S.A.** (NW3460)A

UNIVERSITY OF DURHAM

LECTURESHIP IN ORGANIC CHEMISTRY

Applications are invited for a Lectureship in Chemistry from candidates with research interests and achievements in any area of organic synthesis. In addition to promoting an active research group, the appointee will be expected to give lectures, tutorials and conduct laboratory teaching in various aspects of Organic Chemistry.

The post is available from October 1989. The initial salary is expected to be at an appropriate point on the Lecturer Grade A scale, £9,260–£14,500, but more experienced applicants will receive very serious consideration.

Further particulars may be obtained from the **Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, to whom applications (3 copies), including the names of three referees, should be sent by 14 April 1989.** (8734)A

ASSISTANT PROFESSOR

MOLECULAR PHARMACOLOGY OF ION CHANNELS

Tenure track position with a 12-month state-funded salary, generous laboratory space and start-up funds are available in an established Biochemical Pharmacology Department which is expanding in the area of molecular pharmacology. Applicants should have strong research experience in the areas of ion channels biophysics and/or the application of molecular biological approaches to study channel structure or regulation.

Send curriculum vitae, statement of research interests, and at least three letters of reference before April 21 to **Dr. Linda M. Hall, Chair, Department of Biochemical Pharmacology, School of Pharmacy, 317 Hochstetter, SUNY at Buffalo, Buffalo, NY 14260.**

SUNY is an Affirmative Action/Equal Opportunity Employer. (NW3458)A

QA Standards Development and Validation

£12,000-£17,000
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Wellcome Biotech is at the forefront of the application of biotechnology to the production of new medical products and currently has an opening for a Senior Scientist to lead a small group involved in QA Standards Development and Validation.

Reporting to the Head of the QA Biological Assay Section, you will be engaged in the identification and development of a range of biological assays to ensure the quality of novel therapeutic proteins. The work will be concerned with the functional, clinical and purity aspects of the product and will involve a wide range of immunochemical, tissue culture and virological techniques.

In addition to holding a PhD in a relevant subject, you will have at least 5 years' practical experience and knowledge of immunological and virological assays. An interest in computing and an understanding of statistical analysis would also be an advantage.

In addition to an attractive salary, you can look forward to an extensive range of benefits which includes 5 weeks' holiday, pension scheme, profit share scheme, first-class sports and social facilities and a subsidised restaurant.

If you are interested in this position please write with full career details for an application form, to Alison Baker, Personnel Assistant, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, quoting reference 91/68.

Previous applicants need not apply.

(8781)A

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Wellcome

RESEARCH SCIENTIST PHARMACOLOGY

Amgen is a dynamic, growing organization producing human therapeutic products through recombinant DNA technology. Our leadership in the development of Erythropoietin and Granulocyte Colony Stimulating Factor has placed us at the leading edge of the biotechnology industry. Our continued growth has created an immediate opportunity for a Research Scientist in our Pharmacology Group.

Applicants must possess a Ph.D. in Pharmacology or a related discipline with a strong background in whole animal research and experience in one or more of the following areas: inflammation, wound repair, immunopharmacology, hematology and oncology. Proficiency in statistical analysis of data is essential, and postdoctoral training is highly desirable.

Amgen offers a competitive compensation and benefits package, including equity participation, as well as a challenging and stimulating research environment. Our location one hour northwest of Los Angeles offers the best in Southern California living. Qualified applicants should direct a resume and a list of 3 references, in confidence, to:

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Thousand Oaks
CA 91320

(NW3431)A

AMGEN

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MEDICAL RESEARCH COUNCIL
VIROLOGY UNIT
UNIVERSITY OF GLASGOW

TWO POSTDOCTORAL POSITIONS

1. MOLECULAR BIOLOGISTS

A scientist with experience in protein and/or nucleic acid research is required to work on a project involving Adenovirus genetics and gene regulation.

2. CELL BIOLOGIST/ZOOLOGIST

A scientist with experience in cell biology or animal pathology is required to work on herpes virus pathogenicity and/or latency. This position offers the opportunity for collaborations with molecular biologists, immunologists and geneticists to investigate the specific role of individual virus genes. The complete viral DNA sequence is known providing a firm foundation for this investigation.

Both positions are available for three years on the Non-Clinical Scientific Staff with salary starting at an appropriate point on Grade II (currently £11,070-£14,500 pa). Previous experience with virus systems is advantageous but not essential as training would be provided in well equipped laboratories.

Applicants should send a Curriculum Vitae together with the names and addresses of three professional referees to the **Director, Professor J H Subak-Sharpe, Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR.**

Closing date: 14 April 1989.

The MRC is an equal opportunity employer. (8762)A

MRC

CJA
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Telex No. 887374 Fax No. 01-256 8501

High profile career appointment: opportunity for equity participation and scope for advancement to Main Board in medium term.

NATIONAL POWER **HEAD OF ENVIRONMENTAL POLICY**

CITY £40,000–£45,000 + BONUS + CAR

ONE OF THE TOP TEN GROUPINGS IN THE UK

For this new appointment, applications are invited from graduates, aged 40+, with well established environmental credentials and a substantial record of relevant exposure and achievement (at least 10 years) at senior level in the public sector or industry. Reporting to the Main Board Technical Director, the successful candidate will co-ordinate the development of our client's environmental policy with particular emphasis on fossil fuels, and be responsible for a budget of about £10 million, spent largely on environmental research. Principal tasks will include establishing environmental effects and proposing effective controls, participating in national and international forums, initiating and guiding conservation projects, and developing internal communications and external representations with government bodies and NGOs on key environmental issues. Initial salary negotiable £40,000–£45,000 + performance related bonus, car, contributory pension and free family medical cover. Assistance with removal expenses if necessary. Applications in strict confidence under reference HEP4657/N to the Managing Director: CJA.

CAMPBELL-JOHNSTON ASSOCIATES (MANAGEMENT RECRUITMENT CONSULTANTS) LIMITED, 3 LONDON WALL BUILDINGS, LONDON WALL, LONDON EC2M 5PJ. TELEPHONE 01-588 3588 or 01-588 3576. TELEX: 887374. FAX: 01-256 8501. (8757)A

PETROLOGY-GEOCHEMISTRY OF MINERAL DEPOSITS/AMERICAN MUSEUM OF NATURAL HISTORY

The Department of Mineral Sciences of the American Museum of Natural History invites applications for a tenure-track position as Assistant Curator of petrology and geochemistry of mineral deposits. We seek a creative scientist whose primary rsponsibility will be to carry out a vigorous research program related to mineral deposits. This research may have a structural/tectonic, theoretical or experimental approach to the study of ore deposits, but all should also be sample- and field-oriented. Collaboration with outside investigators to broaden the scope of research is encouraged. It is expected that the curator will supervise Ph.D. students in the AMNH-Columbia-CUNY Ph.D. program in geoscience and will develop relevant courses. The Museum is a repository for important research collections, and the curator is expected to develop significant ore deposit collections. Other duties include participation in departmental activities and occasional development of Museum exhibitions, seminars and courses.

The Department encompasses the fields of mineralogy, petrology, mineral deposits and meteoritics with 5 Ph.D. researchers; post-docs, research associates and support staff help with research and collections. Instrumentation includes a 9-spectrometer ARL-SEMQ microprobe with EDS, a Zeiss digital SEM with EDS, automated powder and single-crystal X-ray diffractometers, fluid inclusion and petrographic microscopes, sample preparation facilities and a network of microcomputers.

Applications should be submitted by May 15; the search will continue until a suitable candidate is found. Salary is competitive depending upon the experience of the candidate. Applicants must have a Ph.D. in hand (or nearly so) and should send a curriculum vitae, a succinct description of research interests and the names of three references to:

Dr. George E. Harlow
Chairman, Search Committee
Department of Mineral Sciences
American Museum of Natural History
Central Park West at 79 Street
New York, NY 10024-5192
(212) 769-5378

An equal opportunity employer. (NW3445)A

POSTDOCTORAL ASSOCIATES

to study cAMP-dependent kinase gene expression and structure/function using recombinant DNA and gene transfer methodologies. Positions available immediately with stipend support of up to $30,000/year.

Send curriculum vitae and three letters of reference to:

Dr. Richard A. Maurer,
Department of Physiology and Biophysics,
University of Iowa College of Medicine
Iowa City, Iowa 52242 U.S.A.

The University of Iowa is an Equal Opportunity/Affirmative Action Employer (NW3449)A

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY
Protein Chemist/Enzymologist

Applications are invited for the post of

Senior Research Assistant

to work with Professor H. H. Rees and Dr. R. Powls on the enzymology and characterisation of reactions involved in the metabolism and regulation of the concentrations of the polyhydroxylated steroidal insect moulting hormones (ecdysteroids). Experience of enzymology or steroid biochemistry would be an advantage, but not essential. Interested candidates with other experience are encouraged to apply.

The appointment is tenable for up to three years from a mutually convenient date as soon as possible. Initial salary within the range of £9,865-£11,680 per annum.

Applications, together with the names of three referees, should be received as soon as possible by **The Director of Staffing Services (AS) The University, P.O. Box 147, Liverpool, L69 3BX**, from whom further particulars may be obtained.

Quote ref. RV/298/N

An Equal Opportunities Employer (8716)A

UNIVERSITY OF GLASGOW
POSTDOCTORAL FELLOW
DEPARTMENT OF BIOCHEMISTRY

A vacancy exists to join an active research group interested in the interactions between the adenylate cyclase, tyrosie kinase and inositol phospholipid cellular signal transduction systems in normal and disease states. Studies are being performed at the cellular, molecular and molecular biological levels. Applicants should either have previous experience in certain of these systems or expertise in either protein purification, cell biology or molecular biology.

The position is on an existing MRC programme grant and is tenable for up to three years as of 1st October 1989. Salary as on the 1A scale for applicants with a PhD.

Informal enquiries to Professor Miles Houslay (041-330-4624) and final applications with CV and names of two referees to **Professor Houslay** at **Molecular Pharmacology Group, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ**, by 7th April 1989. (8760)A

THE UNIVERSITY OF MANCHESTER
TEMPORARY LECTURER IN ANATOMY

Applications are invited, from scienc medical or dental graduates, for th above post, in the Department of Cell ar Structural Biology, which is tenable f one year from a date to be arranged. Th department has responsibility for teac ing topographical anatomy, neur anatomy, histology and embryology medical, dental, paramedical and scien students, including students of th newly-established Honours degree Anatomical Sciences. The persc appointed will be expected to hav experience of teaching (including lectu ing) in one or more of the above are and preference will be given to thos with experience in topographic anatomy. The post holder will b encouraged to participate in the nume ous research activities of the departmer Salary range £9,260-£11,680 p.a. acc ding to age and experience.

Further particulars and application form (returnable by 30th March 1989) from th **Registrar, the University, Manchest M13 9PL. (Tel: 061 275 2028)** Quote re 65/89/NAT.

The University is an equal opportuniti employer. (8738)A

NEW INITIATIVE IN MOLECULAR SCIENCES

Pfizer, one of the world's foremost and highly respected pharmaceutical companies, has opportunities for graduate and post-graduate scientists in a creative research environment.

We are building up a Molecular Sciences Group which will play a major part in our Drug Discovery programmes. We are now seeking to recruit experienced Senior Scientists and Research Assistants in Molecular Biology and related disciplines.

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VECTOR DESIGN AND CONSTRUCTION

- ★ PhD in Biochemistry, Molecular Biology or Microbiology
- ★ Experience with vector construction and biochemical manipulation of DNA.

MICROBIAL AND MAMMALIAN EXPRESSION SYSTEMS

- ★ PhD in Biochemistry, Molecular Biology or Molecular Virology
- ★ 1-3 years post-doctoral experience in biotechnology
- ★ Working knowledge of cellular transfection techniques
- ★ Tissue culture experience

PEPTIDE AND GENE SYNTHESIS

- ★ PhD in Chemistry, Biochemistry, or Molecular Biology
- ★ Experience with automated peptide or oligonucleotide synthesis

PROTEIN PURIFICATION

- ★ PhD in Biochemistry, Protein Chemistry or related field
- ★ Post-doctoral experience with a working knowledge of protein purification from recombinant sources
- ★ Working knowledge of HPLC, affinity, ion-exchange and size exclusion chromatographies

If you are a successful applicant you will have the opportunity to play a major role in co-ordinating drug-discovery strategies in diverse therapeutic areas and eventually be responsible for the day-to-day management of a team of specialists and therefore you should have well-developed supervisory and interpersonal skills. Experience in leading scientists in an industrial research environment would be an advantage.

RESEARCH ASSISTANTS

In addition there will be a number of positions available for graduate Research Assistants within the same disciplines. Candidates for these positions should have the following qualifications:

- ★ BSc or equivalent in Biochemistry, Molecular Biology, Virology or Microbiology
- ★ 1-5 years experience using cell or molecular biology techniques.

This is a particularly exciting time to join Pfizer as we have a number of new therapeutic agents being launched on world markets and others reaching the final stages of their development. We also have a major multi-million pound expansion of our research facilities under construction demonstrating our long-term commitment to research here at Sandwich and enabling our scientists to look forward with confidence to the 1990's and beyond.

If you join us, you will be paid a good salary, be eligible for an annual performance bonus of up to 20% of salary, participate in our excellent employment benefit package of pension, share ownership and private medical insurance schemes and be able to live in one of East Kent's charming towns or villages. We will naturally pay your removal expenses.

If you are interested send a brief c.v. to Mr D O'Callaghan, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ.

Pfizer

(8778)A

DRUG DISCOVERY

Neuroscience Research Division

TEAM LEADER—COGNITION

The selected candidate will lead an interdisciplinary research team developing novel drugs for use in the treatment of CNS neurodegenerative disorders including Alzheimer's disease. The qualified candidate will have an extensive background in radioreceptor binding, receptor-effector coupling mechanisms and isolated cell and tissue assays, plus a broad knowledge of the cognition field. Strong evidence of innovative research and leadership skills are essential. We require a Ph.D. in Pharmacology, Neuroscience or Biochemistry with 5-10 years experience, as well as a solid record of scientific accomplishment in the field. Previous experience in drug development and management of a scientific team or leadership of an academic research laboratory are highly desirable. **Reply to job TL.**

NEUROPHARMACOLOGIST—COGNITION

The qualified candidate will be involved in a multi-disciplinary research program which develops novel drugs for the treatment of cognitive disorders. You must have experience in radioreceptor binding assays, second messenger systems and in vitro cell and tissue assays. A Ph.D. in Pharmacology, Neuroscience or Biochemistry with 2-5 years of industrial or postdoctoral work are required. Communication and supervisory skills are essential. **Reply to job C.**

NEUROPHARMACOLOGIST—PEPTIDES

The qualified scientist will participate in an interdisciplinary research effort to discover novel therapeutic entities based on peptide neurotransmitters. Experience with radioreceptor binding assays, receptor-effector coupling and in vitro systems is essential. A Ph.D. in Pharmacology, Physiology, or Biochemistry is required, along with 2-6 years postdoctoral training. Exceptional communication, interpersonal and supervisory abilities are also necessary. Previous work in pharmaceutical drug development is desirable. **Reply to job P.**

Abbott offers an excellent salary and benefits package including profit sharing and a stock retirement plan. We are located in an attractive suburban area 30 miles north of Chicago.

Abbott representatives will be at the FASEB meeting in New Orleans, March 20th-23rd.

To arrange an interview, or if you will not be able to attend, send your resume indicating job interested in to: Patricia Handy, Corporate Placement, Dept. NAT/3-16, Abbott Laboratories, Abbott Park, IL 60064. Abbott is an Affirmative Action Employer.

ABBOTT LABORATORIES

(NW3463)A

Group Leader

ADVANCED THERAPIES FOR CANCER AND INFLAMMATORY DISEASE.

Celltech is one of Europe's foremost biotechnology companies. Operating from our modern purpose-built facilities in Slough, we are among the leaders in the research, development and manufacture of protein-based drug products. We have built up a portfolio of successful candidate therapies, and are now establishing a fully integrated biopharmaceutical capability.

We are looking for an experienced pharmacologist/immunologist to lead a small group studying the therapeutic potential of our drug products. Researching into the mechanisms of biopharmaceuticals for cancer and inflammatory disease, you will use in-vitro and in-vivo models to evaluate the pharmacological value of reagents for specific therapies.

Aged between 30 and 35, you will be PhD qualified in either Pharmacology, Immunology or Cell Biology. You must have between 3 and 6 years' post-doctoral experience including in-vivo research in pharmacology and immunology, either in academia or industry. A natural and easy communicator, you will have the inter-personal skills to manage a scientific team, as well as the ability to produce original research material.

A highly competitive salary will be supported by a comprehensive benefits package which includes a pension scheme, discounted BUPA and relocation assistance if appropriate.

Please write, with full details of your qualifications and experience to date, to Mrs Jane Smith, BSc C BIOL MI BIOL, at Celltech Limited, 216 Bath Road, Slough, Berkshire SL1 4EN. Please quote Ref: 255.

CELLTECH

(8785)A

RESEARCH SCIENTISTS ANALYTICAL BIOCHEMISTRY

Amgen has immediate opportunities for Research Scientists in Analytical Biochemistry to participate in the development of methods for protein characterization and protein stability using normal and reversed phase HPLC and other analytical techniques.

Qualified applicants for these positions must have a Ph.D. in Biochemistry or a related discipline with an emphasis in analytical methods and instrumentation.

Amgen is a dynamic and growing organization providing an exciting and innovative research environment as well as an attractive location northwest of Los Angeles. **Amgen** also offers an excellent compensation and benefits package.

Qualified candidates should send a c.v., including references, in confidence to:

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CA 91320

Equal Opportunity Employer

(NW3430)A

AMGEN

Scientific Director

A privately-owned French pharmaceutical group of companies which is expanding rapidly in the international sphere, is seeking a Scientific Director who will take responsibility for an established modern research centre with a staff of 200 and an annual budget of around US$20 million.

Reporting directly to the President of the Group, the Scientific Director will co-ordinate the activities of the various on-site research departments with those of collaborative projects in external government, universities and private laboratories.

The Scientific Director will optimise the use of human and financial resources and participate in the establishment and selection of the principal fields of research. You will also manage the integration of research programmes arising from partnerships with other companies.

The present major research interests of the Group are:

- cardiovascular pharmacology
- immunology
- gastroenterology
- new technologies for drug delivery.

To apply you must have acquired sufficient research and management experience for a position of this importance. You will have a PhD in biochemistry or pharmacology, or the equivalent. A qualification in businesss management is desired. Fluency in French and English will be required and a working knowledge of German would be helpful.

You will have already led research projects successfully and have demonstrated managerial capacity. Through your personal qualities and scientific experience you will be able to motivate and direct a large research centre towards the discovery of new products.

The research centre is located in a university town in an extremely pleasant part of France.

The very substantial salary to be offered is in keeping with the importance of this position.

Interested and appropriately qualified persons should write to Media System Limited, Garden House, Cloisters Business Centre, 8 Battersea Park Road, London SW8 4BG, enclosing their curriculum vitae and a photograph. Please quote ref: 1953.

(W5978)A

MEDIA SYSTEM

THE OHIO STATE UNIVERSITY
DEPARTMENT OF PEDIATRICS
is seeking a

Full Time Molecular Biologist

at the **Assistant** to **Associate Professor** level with research interests in human development or the genetics of childhood diseases. This regular tenure track position is open to research scientists with either Ph.D. or M.D. training. Laboratory space and equipment allowance will be offered in the new Wexner Institute for Pediatric Research located at Children's Hospital. The Institute offers excellent opportunities for building an independent research program in areas of oncology, immunology, neurobiology, nutrition, virology or gastroenterology in the pediatric patient. Ample opportunity exists for collaboration with investigators in other departments of The Ohio State University College of Medicine and Biotechnology Center. Contact **M. Sue O'Dorisio, M.D., Ph.D., Professor of Pediatrics, Chairperson, Hematology/Oncology Molecular Biologist Search Committee, Children's Hospital, 700 Children's Drive, Columbus, Ohio 43205.**

The Ohio State University is an Equal Opportunity/Affirmative Action Employer. (NW3456)A

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF OXFORD

POSTDOCTORAL RESEARCH ASSISTANT 1A

BR/286

Applications are invited for the above position, available immediately, to work on an SERC supported project concerning the consequences of structural asymmetry for catalysis of the F0/F1 ATP synthase under the joint supervision of Dr. I. D. Campbell and Dr. S. J. Ferguson.

The project involves the use of isotopic labelling and NMR, and funding is available for up to 2 years in the first instance.

Salary on the scale £9,865-£15,720 depending upon age and experience.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to: **The Administrator.**

An Equal Opportunity Employer. (8728)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD, OX1 3QU

POSTDOCTORAL

Cloning and characterisation of yeast mutants altered in their sensitivities to lethal effects of bleomycins. Background in molecular biology and genetics, cellular biology, or biochemistry. Experience with yeast preferred but not required.

Send curriculum vitae and list of three references to: **Dr. Carol Wood Moore, Department of Microbiology, City University of New York Medical College of Cuny and School of Biomedical Education, Room J-910, City College of CUNY, Convent Avenue at 138th Street, New York, NY 10031.**

An Affirmative Action/Equal Opportunity Employer M/F. (NW3348)A

Post-doctoral position

available in a molecular biology laboratory devoted to the study of acquired and inherited blood disorders (leukemia and hemoglobinopathies). Experience in molecular biology is desirable. Send application with C.V. and names of 3 references to **Dr. D.F. Atweh, SUNY Health Science Center, VA Medical Center (111D), 800 Poly Place, Brooklyn, NY 11209.**

SUNY is an equal opportunity/affirmative action employer (NW3464)A

CAMBRIDGE UNIVERSITY

POSTDOCTORAL MOLECULAR BIOLOGIST

A postdoctoral scientist with experience of nucleic acid or protein manipulation is needed to join an active well-funded group working on bacterial hemolysins. We are particularly interested in the transcriptional activation of hemolysin synthesis and export, and the mechanism by which hemolysin protein is translocated across both bacterial membranes (Nucleic Acids Research (1988) 16: 4789-4800; EMBO Journal (1989) 8: 595-607).

Information may be obtained from **Dr. Colin Hughes (0223 333732)** and applications should be sent to him at the **Cambridge University Department of Pathology, Tennis Court Road, Cambridge CB2 1QP, England.** (8700)A

DIRECTOR OF MOLECULAR NEUROBIOLOGY



Impact products, processes and your career

Hoechst-Roussel Pharmaceuticals Inc., an internationally based pharmaceutical company and world leader in CNS research, has an immediate opening in its Neurosciences Research Group for a Director to set up and head its newly created Molecular Neurobiology Department.

This Department will work in close collaboration with existing innovative drug discovery groups in CNS Pharmacology, Biochemical Pharmacology and Medicinal Chemistry. Emphasis of the group will be on development of methodology and basic knowledge relevant to establishing IN VIVO/IN VITRO models for the evaluation of drugs to treat Alzheimer's disease, schizophrenia and affective disorders. Particular focus will be on identification, isolation, and characterization of genes and gene products associated with these diseases, and the study of gene regulation mechanisms.

Requirements of this position include a Ph.D in molecular biology, neurobiology, biochemistry, or a closely related field with extensive experience in the study of gene expression in the central nervous system. The successful candidate will also have a significant record of research accomplishments in molecular biology and a demonstrated ability to direct a multi-disciplinary research team.

Position is located in our modern research facilities on a 110-acre campus in Bridgewater, New Jersey, convenient to good housing, recreation and educational facilities, and offers a comprehensive salary and benefits package.

Please send resume with salary requirements in confidence to: Nancy Wilkerson, Manager, Human Resources, Building M-303, Department 36, Hoechst-Roussel Pharmaceuticals Inc., Route 202-206, P.O. Box 2500, Somerville, NJ 08876-1258. An Affirmative Action Employer M/F/H/V.

(NW3468)A

Hoechst Celanese

Hoechst

CHAIRPERSON

DEPARTMENT OF HUMAN ANATOM'

The School of Medicine, University of California, Davis, invites applications an nominations for the position of Chai person of the Department of Huma Anatomy. The department currentl comprises 10 tenure-track faculty, housed in a modern laboratory building offers genuine training in cell biology anatomy and related disciplines, and i responsible for the teaching of gross neuro and microscopic anatomy an aspects of cell biology in the medica curriculum. Candidates must have distinguished record of scholarship an professional achievements in the area o cell biology, as evidenced by publication and grant awards. They also must hav leadership and administrative abilities and an interest in the departmental teach ing mission. A committment is required t building a strong independent, extra murally funded research program ir modern cell biology as well as to augmen ting departmental research in this area; ar opportunity to appoint a number of new faculty members is available.

Applicants should submit a letter of application, curriculum vitae, a summary of research goals and the names of five references to: **Hibbard E. Williams, M.D., Dean, School of Medicine, University of California, Davis, CA 95616.**

This position will be "Open Until Filled; applicants must be received by July 31, 1989. The University of California, Davis, is an Affirmative Action/ Equal Opportunity Employer. (NW3478)A

SCIENTISTS

PANLABS is expanding its U.S. laboratory facilities to accommodate rapid growth in the Pharmacology Services Division and the Microbial Products Services Division. Applications for positions in the following areas are currently being accepted:

BIOCHEMICAL PHARMACOLOGY

Ph.D. 2-5 years experience

We have an opportunity for a scientist with a demonstrated record of scientific innovation and independence to participate in the start-up and development of a new biochemical pharmacology laboratory. Responsibilities include implementation, validation and automation of high-throughput assays for drug discovery and characterization, including radioligand binding, enzyme activity, and cellular functional responses. Background in immunopharmacology and industrial experience desirable. The successful candidate will play an important role in determining future research directions and staffing for the lab.

NATURAL PRODUCTS/BIOORGANIC CHEMISTS

Ph.D. 0-5 years experience
MS Scientists 3+ years experience

The successful candidates will be part of a multidisciplinary team including biochemists, pharmacologists and molecular biologists. One of the initial responsibilities of these positions will be to purify small bioactive molecules from fermentation broths using standard chemical procedures in combination with liquid chromatography (HPLC).

PANLABS is a growing U.S. based international company that has provided R & D services to pharmaceutical companies and the fermentation industry worldwide for almost 20 years. Located in the high tech corridor north of Seattle, Panlabs offers excellent salaries and benefits. Please send CV with names and phone numbers of three references to:



PANLABS, INC.

Personnel Manager, (SC)
11804 North Creek Parkway South
Bothell, Washington 98011

An Equal Opportunity Employer

(NW3465)A

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF MICROBIOLOGY

TWO RESEARCH ASSOCIATES

Molecular biology/Immunology
Bacterial virulence/vaccines

Applications are invited from graduate or post-doctoral researchers to work with a multidisciplinary Microbial Pathogenicity-Immunology Group, studying the molecular biology of bacterial virulence and vaccine design. One candidate will work with a team studying the design and construction of defined epitope vaccines that incorporate both defineds B-cell and T-cell epitopes and that can be delivered to the host by live delivery systems. The second candidate will study structure-function relationships in bacterial protein toxins, particularly staphylococcal enterotoxins. Experience with recombinant DNA, protein engineering and immunological techniques could be an advantage, but is not essential as training will be provided. One appointment is for two years and seven months, and the second for three years. Further details can be obtained by 'phoning Dr M Kehoe on (091) 222 8143.

The appointments will be made at appropriate points on the Grade IA (£9,865-£15,720 p.a.) or Grade IB (£8,675-£11,680 p.a.) scales, depending on qualifications and experience. Applicants should send a c.v., including the names and addresses and phone numbers of 3 referees, to **Dr M Kehoe, Department of Microbiology, Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, England,** not later than 31st March 1989. (8724)A

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London)

RESEARCH TECHNICIAN

Applications are invited for a post in the Mucocutaneous Biology Research Group to investigate the diversity and stability of fibroblast phenotype. Previous experience in tissue culture, hybridoma technology or immunocytochemistry is desirable.

The appointment is for up to 3 years and the starting salary will be £11,515 per annum.

For further information contact either Dr I M Leigh (01-377 7749) or Dr D M Williams (01-377 7060).

Applications to The Assistant Secretary, The London Hospital Medical College, Turner Street, London E1 2AD. (8739)A

UNIVERSITY OF CANTERBURY

New Zealand

SENIOR LECTURER OR LECTURER IN MOLECULAR BIOLOGY

(Department of Plant and Microbial Sciences)

pplications are invited for a posi-n, as above. Applicants should ve a Ph.D. degree in a relevant ld and an interest in plant olecular biology and plant viro-gy. The appointee will be expec-d to contribute to the teaching of urses in Biochemistry, Molecu-r Biology, Molecular Genetics d Virology and to pursue an tive research programme inclu-ng the supervision of M.Sc. and .D. students.

The salary for Senior Lecturer is a scale from NZ$49,000 to Z$57,000 per annum and a range om NZ$59,000 to NZ$63,000 per num; and for Lecturers is on a ale from NZ$35,000 to Z$42,500 (bar), NZ$44,000 to Z$46,000 per annum.

Further particulars and Condi-ons of Appointment, quoting osition No. PL1, may be btained from Appointments 36117), Association of Common-ealth Universities, 36 Gordon quare, London WC1H 0PF.

Applications close with: A. W. layward, Registrar, University of anterbury, Private Bag, Christ-hurch, New Zealand, on *28 April 989.* (W5968)A

UNIVERSITY OF GLASGOW

NEUROSCIENCE RESEARCH

Research Assistant

required to join a well re-sourced group investigating neurotransmitter receptor dynamics in relation to Alz-heimer's disease using quanti-tative autoradiography and in situ hybridisation.

Applicants should have a back-ground in pharmacology, physiology or biochemistry and hold or expect to obtain a good Honours degree in an appropriate discipline. Appli-cants should be committed to developing a career in neuro-science and will be encouraged to register for a Ph.D.

Applicants, comprisings C.V. and the names and addresses of two referees should be sent to **Professor J. McCulloch, Wellcome Neuroscience Group, University of Glasgow, Garscube Estate, Bearsden Road, GLASGOW, G61 1QB** by 21st April, 1989. (8761)A

IMMUNO

AKTIENGESELLSCHAFT FÜR CHEMISCH-MEDIZINISCHE PRODUKTE

Wir sind ein weltweit erfolgreiches pharmazeutisches Großunternehmen und erzeugen anerkannt hochwertige biologische Arzneimittel.

Hohes fachliches und persönliches Niveau unserer Mitarbeiter sowie intensive Forschung und Innovation stellen für uns die Grundvoraussetzung für eine erfolgreiche Behauptung unserer Führungsposition auf internationalen Märkten dar.

Zur Mitarbeit in unserem Forschungsbereich suchen wir eine(n)

Biochemiker/-in

mit langjähriger Erfahrung auf dem Gebiet der Proteinchemie und Enzymkinetik.

Ihre Hauptaufgabe wird in der selbständigen Durchführung biochemischer Forschungsarbeiten mit Hilfe chemischer und physikalischer Methoden (Affinitätschromatographie, Proteinreinigung) bestehen.

Biochemische Erfahrung auf dem Gebiet der Gerinnungs- und/oder Fibrinolysefaktoren wäre besonders wünschenswert.

Wir bieten eine vielseitige und anspruchsvolle Tätigkeit in unserer Wiener Zentrale. Die Dotierung entspricht internationalen Maßstäben.

Bei näherem Interesse stehen wir für ein informatives Gespräch gerne zur Verfügung bzw. laden wir Sie zur Übermittlung Ihrer Bewerbung ein (Ref. 217).

IMMUNO AG, Personalwesen, Fr. Walla,
A-1220 Wien,
Industriestraße 67,
Tel. (0043/222) 2300/2002 DW

(8783)A

IMMUNO

UNIVERSITY OF SOUTHAMPTON

CRC MEDICAL ONCOLOGY UNIT — GROWTH FACTOR PROJECT

Experienced **cell biologist/biochemist** to take major role in leading group studying involvement of polypeptide growth factors in the growth and dissemination of cancer cells. The unit contains a group actively studying the contribution of EGF/TGF and to tumour cell growth and is investigating generation and use of EGF-receptor antagonists as potential chemotherapeutic agents.

Appointment in the first instance for 3 years with salary up to £15,720.

Applications to **Ms J A Doyle, Staffing Section, The University, Southampton, SO9 5NH.** Further details may be obtained from Dr Donna Davies tel. 0703 777222 Extn. 4296/7.

Please quote reference 269/JAD/jm.

(8737)A

A RESEARCH ASSOCIATE

position is available immediately to study the mechanisms of transformation in human breast epithelial cells, mainly the interaction of carcinogens with oncogenes. Research experience in Molecular Biology is required.

Send curriculum vitae to **Dr. Jose Russo, Department of Pathology, Michigan Cancer Foundation, 110 East Warren Avenue, Detroit, MI 48201.**

(NW3455)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF EARTH SCIENCES

Applications are invited for a Temporary Lectureship, tenable in the first instance for two years from 1st October 1989. The fields of interest of the candidates should be in *Structural Geology* or *Petrology*.

The *Structural Geologist* would be expected to complement existing research areas which include fault analysis, kinematics of thrust zones and microstructures.

The *Petrologist* would complement existing research interests in medium-to-high temperature fluid-rock interactions which would involve the use of stable isotope techniques.

The successful candidate in either field will be extensively involved in field and practical teaching.

Initial salary within the range £9,260-£13,365 per annum on the Lecturer A scale.

Applications, together with the names of three referees, should be received not later than 17 April 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3BX,** from whom further particulars may be obtained.

Quote ref. RV/300/N.

An Equal Opportunity Employer (8715)A

UNIVERSITY COLLEGE LONDON

WITCHWEED RESEARCH AT UCL

The post of

POSTDOCTORAL PLANT PHYSIOLOGIST

is available for work on the parasitic weed Striga. The project is funded by Overseas Development Natural Resources Institute for two years, and is to investigate the effects of Striga on the Growth and Physiology of Maize.

Salary range £11680-14500 pa plus LW of £1650.

Further details and c.v. plus 2 references to **Professor G R Stewart, Department of Biology (Darwin Building), University College London, Gower Street, London WC1E 6BT,** or telephone **01-387 7050 ext. 2653.**

Equal Opportunities Employer

INTERNATIONAL CENTRE FOR MEDICAL RESEARCH (CIRMF)

Franceville, Gabon

CIRMF currently employs 165 people of 10 nationalities. It posseses well-equipped laboratories of immunoparasitology, microbiology, endocrinology, epidemiology and clinical biology; an excellent primatology centre and a good library.

A RESEARCH ASSISTANT/TECHNICIAN

is required in immunoparasitology. The candidate should possess either technical or graduate qualifications and have several years experience in modern immunological methods (monoclonal antibody production, cytotoxicity rests and basic immunochemical techniques). She/he would work in close collaboration with the head of the department, Dr. Margaret Pinder, and general duties will include maintenance of laboratory supplies.

The appointee would be expected to commence by September 1989. A 2-year contract (renewable) is offered which includes an attractive salary (based upon qualifications and experience); relocation, and expatriation allowances, housing on the 80 acre CIRMF campus and 60 days' paid annual leave. Ability to speak French would be an advantage.

Letters of interest will be held in confidence and should include detailed CV, publications list, and the names and addresses of 3 referees. Send this information to **Dr. G. E. Roelants, Deputy Director General, CIRMF, BP 769, Franceville, Gabon**, or telephone (241) 677132 or telex 6708 CIRMGAB or telefax (241) 677295 for further information. (W5979)A

MEDICAL RESEARCH COUNCIL
Institute of Hearing Research
POSTDOCTORAL BIOLOGIST

The Biology section of the Institute of Hearing Research has a new position for a post-doctoral scientist to work on hereditary deafness in mice. The project involves structural and functional studies of the inner ear during development. One particular interest of the section is the role of melanocytes in the development and function of the inner ear. The Biology section has good facilities for single unit and gross electrophysiology, light and electron microscopy, excellent computing and workshop support, and in addition has collaborative links with molecular genetics laboratories for genetic studies of deafness. Experience in electrophysiology, electron microscopy or molecular biology would be an advantage, but is not essential.

For further details about the projects available contact **Dr. K.P. Steel, MRC Institute of Hearing Research, University of Nottingham, University Park, Nottingham NG7 2RD, U.K. Telephone: 0602 223431. Fax 0602 423710.**

The position is a short term appointment for three years at a salary in the range £9,260 to £14,500 (Non-Clinical Scientific Grade II), starting preferably in October 1989. (8733)A

UNIVERSITY OF OXFORD
INORGANIC CHEMISTRY LABORATORY
SENSORS RESEARCH

Two Postdoctoral positions will be available in the Oxford Sensors Centre to work on (a) redox active ionophores (a.s.a.p.) and (b) enzyme electrochemistry from September 1989.

Salary will be on the Research IA scale.

Applications should be submitted to the **Administrator, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR.**

The University is an Equal Opportunity Employer

(8746)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PHYSICS
LECTURER
(3 POSTS)

The University of Leeds expects to be able to make the following three appointments in the Physics Department with effect from 1 September 1989:

N.A.A.S. POST

One lecturer in the field of experimental ultra high energy gamma ray astronomy, based at Haverah Park (Reference number 52/45).

INTERDISCIPLINARY RESEARCH CENTRE POSTS

Two temporary lectureships, each for a fixed term of two years, which will become available as a consequence of the establishment of an IRC in Polymer Science at Leeds. Applicants for these posts should have research interests in Condensed Matter Physics (52/48) or Astronomy and Astrophysics (52/47).

Salary for each post will be on the Lecturer Grade A scale (£9,260 – £14,500) according to qualifications and relevant experience.

Application forms and further particulars may be obtained from, and completed applications forwarded to, the Registrar, the University, Leeds LS2 9JT (Tel (0532) 333965 – direct line), quoting the appropriate reference number. Closing date for application 20 April 1989. (8763)A

MASSEY UNIVERSITY

Palmerston North, New Zealand

POSTDOCTORAL POSITION IN PROTEIN CRYSTALLOGRAPHY

Applications are invited for a Postdoctoral position in protein crystallography with an established group in the Department of Chemistry and Biochemistry.

The project is aimed at the determination of the three-dimensional structures of heat-stable enzymes by x-ray crystallography through the preparation of diffraction-quality crystals of enzymes and subsequent x-ray structure analysis. Mutants of several of the enzymes are currently being prepared. The appointee will also have the opportunity to participate in other protein structural projects in the laboratory.

Applicants with experience in protein crystallography are preferred, but small-molecule crystallographers or biochemists with an interest in protein structure are also invited to apply.

Facilities include CAD4 diffractometer, Evans and Sutherland PS330 interactive graphics system, Optronics microdensitometer, and rotating anode generator.

The position, which is for a period of up to two years, is available immediatley. Further information may be obtained from Dr E N Baker at the University.

Salary: NZ$35,000.

Applications, including a curriculum vitae, a list of relevant publications and the names and addresses of three referees, close with Mrs V B Bretherton, Personnel Section, Massey University, Palmerston North, New Zealand, on *30 April 1989*. (W5976)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS CENTRE FOR APPLIED MICROBIOLOG & RESEARCH

Division of Pathology
Legionella Section

BASIC GRADE MICROBIOLOGIST

An opportunity exists for a grad ate to join this active group stud ing all aspects of the ecolog survival and pathogenicity Legionella pneumophila, t cause of Legionnaires' diseas The appointee will study the ef ciency of drift eliminators preventing the release of micr organisms from cooling towe Harmless biological tracers a various air sampling techniqu will be utilised in conjunction wi a test rig construction on site.

The position is available fr 1st April, 1989 for a period of months at the level of Basic Gra Microbiologist (£8,255-£11,0 per annum).

A science degree is require preferably with some microbio gical component or experience.

Further information may obtained from Dr J. W. Lee.

NHS terms and conditions v apply.

Application forms can be tained from the Personnel Offic PHLS Centre for Applied Mic biology & Research, Port Down, Salisbury, Wilts. SP4 0J Tel: (0980) 610391 Ext. 240, whom completed forms should returned by 31st March, 1 quoting Post No. 0711. (8751)A

RESEARCH ASSOCIATES

COR Therapeutics, Inc. is a recently established and rapidly growing biotechnology company located in the San Francisco Bay Area. We specialize in the development of novel therapeutics for cardiovascular disease. We are actively recruiting highly qualified research associates to join our Research and Development Departments.

Research Associates are sought in Molecular Biology, Protein Biochemistry, Cell Biology, and Monoclonal Antibody Production.

These positions offer the opportunity to study basic mechanisms of receptor ligand interactions and to use this information to design therapeutic agents effective in the treatment of several types of cardiovascular disease. Preference will be given to individuals with prior research and/or laboratory experience.

COR Therapeutics, Inc. offers competitive salaries, benefits and attractive equity positions to its employees combined with the challenge and opportunity to make a significant contribution to this new organization. To apply, please send *curriculum vitae* to COR Therapeutics, Inc., 256 E. Grand Avenue, Suite 80, South San Francisco, CA 94080, ATTN: Human Resources. EOE

(NW3477)A

COR Therapeutics, Inc.

UNIVERSITY OF CAMBRIDGE

University Assistant Lecturer
in the Department of Anatomy

Applications are invited from suitably qualified graduates, for the post of a University Assistant Lecturer in the Department of Anatomy to hold office from 1 October 1989 or as soon thereafter as possible. The Department has extensive and well-funded facilities for research, in various branches of neuroscience including brain research, neuroendocrinology and behavioural studies, molecular cellular and developmental biology.

University Assistant Lecturer in Biochemistry

Applications are invited for a University Assistant Lectureship in the Department of Biochemistry to hold office from 1 October 1989 or as soon thereafter as possible. Candidates should hold a PhD degree, or the equivalent, and have an outstanding record in research and enthusiasm for teaching.
Candidates with postdoctoral experience and interest in any area of biochemistry are welcome to apply.

Salary range: £10,460 to £14,500

Further information and application form from:
The Secretary, Appointments Committee, 19 Trumpington Street, Cambridge, CB2 1QA (Tel: 0223 334999 Fax: 0223 332355).
Closing date: 15 April 1989

(8712)A

INSTITUTE OF CANCER RESEARCH
Sutton, Surrey

TECHNICIAN

There is a vacancy for a **TECHNICIAN** to work in the **FLOW CYTOMETRY LABORATORY**. The laboratory runs a number of collaborative research projects with other teams in the Institute. It also provides a general service in the field of flow cytometry and cell sorting. This is one of the leading flow cytometry facilities in the UK and the post offers the right applicant an opportunity to gain expertise in a rapidly expanding field.

The post would suit someone with a degree (or equivalent) in a biological science. Previous experience in flow cytometry, although an advantage, is not essential as training will be provided.

Salary in the range £8172-£10905 (all inclusive).

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Any enquiries should be addressed to Dr. M. G. Ormerod, 01-643-8901 ext. 4694.

Please send applications, in duplicate, with the names and addresses of two referees to **The Personnel Officer, the Institute of Cancer Research, 17a Onslow Gardens, London SW7 3AL** quoting reference number 3.89.T.N.84.

FUNDED BY THE CANCER RESEARCH CAMPAIGN.

(8735)A

Department of Scientific and Industrial Research

DSIR

IMMUNOLOGISTS/ BIOCHEMISTS

Biotechnology Division

The Immunology Group of the Biotechnology Division, Department of Scientific and Industrial Research has two openings for immunologists/biochemists. The appointees will be required to extend existing programmes in monoclonal antibody production, including development of monoclonal antibodies for diagnostic purposes and methods for large scale animal cell culture.

The successful candidates will have proven expertise in the fields of immunology/biochemistry. Specific skills in hybridoma technology, development of immunoassays, in vitro cell culture techniques, animal cell metabolism and protein biochemistry would be an advantage.

The positions are initially for a period of 3 years with the possibility of further extension for appropriate candidates.

Salary will be commensurate with qualifications and experience.

Please apply in writing, enclosing a curriculum vitae which should include the names of two referees, by Friday, 31 March 1989, and post or facsimile to:

The Personnel Officer,
DSIR, Private Bag,
Palmerston North,
NEW ZEALAND.

Fax: 0064 63 61130

An equal opportunity employer.

(W5977)A

M.D. ANDERSON CANCER CENTER

Postdoctoral and Research Associate Positions, M.D. Anderson Cancer Center, Department of Clinical Immunology and Biological Therapy in the Laboratory of Cytokine Research. Identification of new cytokines (growth inhibitors and stimulators) produced by the immune system and involved in tumor cell surveillance. Interested in understanding the role of oncogenes, signal-transduction and growth factors in the mechanism of tumor cell resistance to these cytokines. Experience in cell biology, protein purification or gene cloning; background in cellular immunology and/or tumor biology is desirable. Ph.D. and/or M.S./B.S.

Please send C.V. with the names of three references to: **Dr. Bharat B. Aggarwal, Chief, Section of Cytokine Research, M.D. Anderson Cancer Center, Department of Clinical Immunology and Biological Therapy, 1515 Holcombe Boulevard, Houston, Texas 77030.**

EOE/AA

(NW3479)A

POST-DOCTORAL SCIENTISTS : MOLECULAR GENETICS AND CELL BIOLOGY

CANCER RESEARCH CAMPAIGN HUMAN CANCER GENETICS RESEARCH GROUP

UNIVERSITY OF CAMBRIDGE

Three post-doctoral positions will become available from early summer 1989 in this newly-established research group funded by the CRC. The group will eventually comprise 15-20 benchworkers, and will be sited in new laboratories within the Department of Pathology close to other groups involved in molecular genetics, biochemistry and developmental biology. The group has a broad interest in inherited predisposition to cancer in man, but is focussed on the cloning and biology of the gene for multiple endocrine neoplasia, type 2. Each of the three scientists to be appointed will be responsible for a small team working jointly on this project. Two posts (one for a molecular geneticist, the other for a cell or developmental biologist) will be at a senior level; one (requiring some experience in molecular genetics) will be suitable for a first or second post-doc appointment. Contracts 3 years (junior post-doc) or 5 years with possibility of renewal; salaries on University scales £9,865-£19,310 pa. according to age and experience.

The University of Cambridge is an equal opportunities employer.

Applications, with the names and addresses of two referees should be sent to Dr. B.A. Ponder, who is presently located at: **Institute of Cancer Research, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG.**

Informal enquiries encouraged: phone Bruce Ponder on 01-643 8901 ext. 4693. (8736)A

UNIVERSITY OF NOTTINGHAM SCHOOL OF AGRICULTURE

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL SCIENCE
AFRC Research Group on Hormones and Farm Animal Reproduction

Research Technician Grade 3

Applications are invited for the above post within the Department of Physiology and Environmental Science. The successful applicant will be part of a group studying the trophoblast factors involved in the maternal recognition of pregnancy in ruminants and will be required to carry out the radioammunoassay of hormones.

In addition, there may be a requirement to handle domestic animals.

This research post is funded until 31st December 1989 in the first instance, with probable renewal, and has a current salary scale £6,636 to £7,828 per annum for a 5 day, 37 hour working week.

Application forms are obtainable from: **The Administrative Assistant, University of Nottingham, School of Agriculture, Sutton Bonington, Nr Loughborough, Leics, LE12 5RD. Tel: Nottingham 484848 Ext: 8302.** Please quote Post Ref 89/06. (8698)A

POSTDOCTORAL POSITION

Available immediately for cell and molecular biologist to participate in a comprehensive study examining DNA repair mechanisms and cellular recovery processes and their relationship to radiation resistance in cultured insect cells. Emphasis will be on identification of DNA and proteins associated with the pronounced radioresistance. Experience in modern molecular biology techniques desirable. Please submit curriculum vitae and names of three references to:

Dr. T. M. Koval, Department of Oncology, Mayo Clinic, Rochester, MN 55905.

An equal opportunity/affirmative action employer. (NW3459)A

INSTRUCTOR POSITIONS IN MICROBIOLOGY

Responsibilities are to generate monoclonal antibodies against human lymphocytes. M.D. required and at least 2 years of postdoctoral training. Expertise in monoclonal antibody technology, the use of assays to screen antibodies, and immunochemical experience to purify and characterize such antibodies are required.

Submit curriculum vitae, description of research interests and names of 3 references to **Dr. Jonathan Uhr, Dept. Microbiology U TX Southwestern Med. Ctr., 5323 Harry Hines Blvd., Dallas, TX 75235-9048** by April 30, 1989. *UT Southwestern Med. Ctr. is an Equal Employment Opportunity institution.* (NW3475)A

PESTICIDE TOXICOLOGIST/ENTOMOLOGIST

The Department of Entomology, Washington State University invites applications and nominations for a Research, tenure-track, Assistant Entomologist Position located at Puyallup, WA. Conduct research on the movement of pesticides in the vegetable crop ecosystem of Western Washington (additional information available upon request). Ph.D. required, preferably in Entomology.

Candidates with Ph.D. in Toxicology (or a related science) with expertise in pesticide toxicology will also be considered.

Applications accepted through June 1, 1989. Send letter of application, statement of professional goals and research plan, curriculum vitae including a list of all publications, descriptions of research experience and goals, and a list including phone numbers of 5 referees to:

John J. Brown, Chair Search Committee, Department of Entomology, Washington State University, Pullman, WA 99164-6432. WSU IS AN EO/AA EDUCATOR AND EMPLOYER. Protected group members are encouraged to apply. (NW3442)A

POSTDOCTORAL POSITION IN MOLECULAR PARASITOLOGY

Available immediately for studies on the mechanisms of action of serveral antimalarial drugs, including recently recognized in this laboratory as the prototype of a new class of anti-malarial agents. A strong background in Biochemistry and Enzymology, with some knowledge of molecular genetics is required.

Further details are available and candidate should send curriculum vitae and names of three references to:

Prof. Richard M. Franklin, Biocenter — University of Basel, Klingelbergstrasse 70, CH-4056 Basel/Switzerland. (W5973)A

AFRC INSTITUTE FOR GRASSLAND AND ANIMAL PRODUCTION

Welsh Plant Breeding Station
Aberystwyth

HIGHER SCIENTIFIC OFFICER
(Temporary Appointment)

Applications are invited for a Higher Scientific Officer in the Grassland Agronomy & Ecology Department to establish guidelines for the management and nutrition of South American Camelids in relation to reproductive performance and fibre production under UK conditions. The temporary post is funded by a MAFF Open Contract.

Qualifications
First or upper second class honours degree in Agricultural Science with at least two years' post graduate experience in animal husbandry/nutrition.

A driving licence is essential.

Terms & Conditions
Salary scale: £10,026-£13,460
Non-contributory superannuation scheme.
Annual leave: 25 days plus 10.5 Public & Privilege holidays per annum.

Written requests for further particulars and application forms should be made to the Secretary, AFRC Institute for Grassland and Animal Production, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed, SY23 3EB (Quote Ref. 89/7)

Closing date: 3 April 1989

The AFRS is an Equal Opportunity Employer. (8723)A

Amended Advertisement

UNIVERSITY OF CANTERBURY

New Zealand

SENIOR LECTURER OR LECTURER IN MICROBIOLOGY

(Department of Plant And Microbial Sciences)

Applications are invited for th above position. Applicants shou have a Ph.D degree in Micr biology. Preference will be giv to a candidate with research expe ience in non-medical microbiolo and microbial metabolism. Th appointee will be expected to co tribute to the teaching of micr biology courses at all levels ar first-year biochemistry, and pursue an active research pr gramme including the supervisic of M.Sc. and Ph.D. students.

The Salary for Senior Lecture is on a scale from NZ$49,000 NZ$57,000 per annum and a rang from NZ$59,000 to NZ$63,000 p annum, and for Lecturers is on scale from NZ$35,000 NZ$42,500 (bar), NZ$44,000 NZ$46,000 per annum.

Further particulars and Cond tions of Appointment, quotir Position No.PL2, may be obtain from Appointments (36015 Association of Commonweal Universities, 36 Gordon Squar London WC1H 0PF.

Applications close with: Th Registrar, University of Cante bury, Private Bag, Christchurc New Zealand on 31 May 1989. (W5982)A

THE UNIVERSITY OF MANCHESTER DEPARTMENT OF CELL & STRUCTURAL BIOLOGY IN COLLABORATION WITH ICI PHARMACEUTICALS ALDERLEY PARK

POSTDOCTORAL RESEARCH ASSISTANT and **PREDOCTORAL RESEARCH ASSISTANT** required for a 3 year research project, supported by the ICI Strategic Research Fund, to investigate extracellular matrix molecules and their receptors in the control of extracellular matrix biosynthesis, cell behaviour and cell differentation in the developing mammalian palate. The project involves a variety of developmental, cell biological and biochemical techniques. Applicants should preferably have a background or expertise in at least one of these areas ie. biochemistry, cell biology, developmental biology. This fundamental project is part of a larger research effort within the department focussing on cell-matrix interactions in development and disease. There is a stimulating research environment, critical mass of personnel and well equipped laboratories. The project involves considerable liaison and collaboration with equally active and well equipped research groups at the nearby ICI Pharmaceuticals Research Laboratories. The posts are available immediately. Salary range £11,680-£15,720 p.a. (Associate) and £10,460-£11,680 p.a. (Assistant) depending on age and experience. Superannuation. Applications including full curriculum vitae and the names and addresses of two referees to Professor Mark W.J. Ferguson, Department of Cell & Structural Biology, the University, Manchester M13 9PL. (Tel: 061 275 6775). Closing date is March 31st, 1989. The University is an equal opportunities employer. (8697A)

FLUID FLOW MODELLER

The sandstone cementation, cation transport, heat and fluid movement in the 360 Myr old sedimentary basin of N. England is being researched by three Ph.D students. A central part of the project is to quantify mass transfer of minerals, heat and soil by **developing computer models** of ancient compactional fluid flow or diffusion through subsiding porous rock. This modeller will need a Ph.D, or a BSc. plus relevant experience, formal geological training is not necessary. Based at Glasgow University, with excellent VAX and SUN computing in Dept. Salary 3 yrs on 1A scale starting £11,015. Apply by 4 weeks after publication date to **Dr. R. S. Haszeldine, Applied Geology, Glasgow G1 1XJ. Scotland. Tel. 041-552-4400 ext 2148.** (8695)A

OVER HALF A MILLION SCIENTISTS

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UNIVERSITY COLLEGE OF SWANSEA

Lectureship in Molecular Genetics

Applications are invited for the vacancy of Lecturer Grade A in Molecular Genetics in the School of Biological Sciences. This is an appointment approved under the new Academic Appointments Scheme. The School of Biological Sciences is a large, well equipped and progressive department and offers a suitable candidate with a good research record, the opportunity to develop an active laboratory and to teach at all levels.

The apppointment will date from October 1, 1989 and will be made in the middle range of the scale: £9,260-£14,500 per annum together with USS/USDPS benefits, if required.

Informal enquiries may be made to Professor J A Beardmore, School of Biological Science (Tel 0792 295382: Fax 0792 295447) but further particulars and application forms (2 copies) must be obtained from the Personnel Office, University College of Swansea , Singleton Park, Swansea, SA2 8PP, to which office they should be returned by **Friday, April 14, 1989.** (8696)A

Saudi Arabia

The **King Faisal Specialist Hospital and Research Centre** in Riyadh, Saudi Arabia has fully equipped research laboratories. The objective of the **Research Centre** is to provide facilities for research into a broad range of disorders particularly those prevalent in Saudi Arabia. The Research Centre collaborates closely with the clinical departments in the 500 bed technically advanced hospital.

The DEPARTMENT OF BIOLOGICAL AND MEDICAL RESEARCH is currently seeking highly qualified individuals for the following positions:

SCIENTIST I - Ph.D. with 15 years experience and demonstrated international scientific distinction in immunochemistry research. Will head active research on immunochemical aspects of airborne allergens, tumor immunology, infectious diseases and immunological aspects of virology.

SCIENTIST III - Ph.D. with 5 years experience with a particular interest in oncogenes. Will work with the molecular biology group.

SCIENTIST III - Ph.D. with 5 years experience in the biochemistry of endocrine disorders. Will work on cancer and disease of the thyroid gland.

SCIENTIST III - Ph.D. with 5 years experience in the field of vitamin D metabolism and metabolic bone disease. Will work on disorders of calcium metabolism.

SCIENTIST III - Ph.D. with 5 years experience in biochemistry. Will work in research of diabetes mellitus.

Several positions available for:

RESEARCH ASSISTANTS - Ph.D. with 1-2 years experience with evidence of scientific achievement. Will support research in cancer, immunological disorders, infectious diseases, parasitic disorders, inborn errors of metabolism, metabolic bone, diabetes and thyroid disease.

TECHNICIANS - BS or MS with 3 years experience. Will support research mentioned above. Also, positions for technicians with training in microbiology, cardiovascular physiology, pharmacokinetics and analytical biochemistry.

The substantial tax savings and attractive benefits for a 24 month contract include:

- Relocation allowance and free furnished housing
- 7 day post leave and 13 holidays during each year of contract
- 30 day annual leave with a round trip ticket to point of origin
- Educational leave for Ph.D.'s
- Free medical and dental care and emergency leave
- Educational assistance for eligible dependent children

For further information and/or to apply, please send a detailed resume to: **HCA International Company, Dept. N-0316, P.O. Box 550, Nashville, TN 37202-0550** or call toll free **1-800-251-2561**, in London: **HCA International Ltd., 49 Wigmore St., London, England W1H 9LE** or call **935-7185.** HCA is an Equal Opportunity Employer.

HCA International Company

(NW3483)A

ASSOCIATE/FULL PROFESSOR OF CONSERVATION BIOLOGY AND ECOSYSTEM MANAGEMENT

The University of Michigan, School of Natural Resources is seeking applicants for the position of Associate/Full Professor of Conservation Biology and Ecosystem Management.

Nine month, tenure-track research and teaching position with leadership responsibility of this new field of study. Positions may be filled by a person with PhD and primary training in either Biological or Social Sciences. However, if PhD and experience are in Social Sciences, the applicant must have one degree in Biology. Regional or national experience and commitment to this field are required together with demonstrated research performance commensurate with the appointment level.

The School of Natural Resources is an interdisciplinary, research-oriented professional school focusing on the development of policies and management programs promoting the protection and sustained use of renewable natural resources, and on the training of practitioners and researchers these ends.

Send curriculum vitae, statement of research interest, list of publications, transcripts of college work, and names of three persons able to evaluate leadership, research, and teaching skills to:

Dr. James E. Crowfoot, Dean, The University of Michigan, School of Natural Resources, 3512 Dana Building, Ann Arbor, MI 48109-1115.

This position will remain open until filled. First review of applicants on April 14, 1989. This position is subject to availability of funds. *The University of Michigan is a non-discriminatory, affirmative action employer.* (NW3453)A

POSTDOCTORAL POSITION IN MOLECULAR VIROLOGY

We are seeking a recent Ph.D. with strong background in molecular biology to join six investigators studying the molecular basis of herpes simplex and varicella zoster virus pathogenesis. Prior experience in virology is not required. Salary range: $21,000 to $25,000. Submit curriculum vitae and brief description of prior research to:

Lawrence Stanberry M.D., Ph.D.,
Infectious Diseases,
Children's Hospital Research Foundation (CHRF),
University of Cincinnati,
Elland and Bethesda Avenues,
Cincinnati, OH 45229.

CHRF is an Equal Opportunity/Affirmative Action Employer. (NW3444)A

神戸大学医学部解剖学第2講座
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1989年3月31日付で現教授停年退職致しますのでその後任の主任教授を広く公募中。
新しい領域の研究者を望みますが、教育は解剖学両講座で均等に分担します。
問合せは　〒650 神戸市中央区楠町7-5-1
神戸大学医学部長へ：
Fax 78-351-6709
Tel 078-341-7451 (ext 3030) (W5981)A

CARDIOVASCULAR FACULTY POSITION

Applications are invited for a tenure-track faculty position in the area of Cardiovascular Physiology and Control of Circulation at the level of Associate or full Professor. Candidates must have a Ph.D. or M.D. degree and sufficient post-doctoral teaching and research experience commensurate with the expected level of appointment. This position will complement an interactive group of faculty with strong commitment to the study of cellular and integrative aspects of neural and endocrine control of physiological systems. The successful candidate will be expected to develop and maintain a strong independent research program and to participate in teaching the medical and graduate students. Interested applicants should submit by June 1, 1989 curriculum vitae, statement of research goals and accomplishments, summary of teaching experience, and the names of four references to:

Esmail Koushanpour, Ph.D.
Chairman, Search Committee
Department of Physiology
Northwestern University Medical School
303 East Chicago Avenue,
Chicago, Illinois 60611
Telephone: (312) 908-8220/7991

Northwestern University is an Affirmative Action/Equal Opportunity Employer. Hiring is contingent upon eligibility to work in the United States. (NW3437)A

THE UNIVERSITY OF SUSSEX
LECTURER IN SPACE PLASMA PHYSICS

Applications are invited from men and women with a background in plasma physics, and proven research expertise, for a Lectureship in Experimental Physics from 1st October 1989, for which a bid has been made to the UGC under the New Academic Appointments Scheme. It is intended that the age of the appointee should be significantly under 35.

The Space and Plasma Physics group at Sussex is involved in a range of well-funded collaborative projects concerned with both spacecraft-borne experiments, eg AMPTE, CRRES and CLUSTER missions, and ground-based experiments, eg the European EISCAT auroral radar project and related optical work. The group's interests include data analysis/interpretation, computational/simulation and development of novel instrumentation.

Salary will be within the Lecturer Grade A scale £9,260 to £14,500 per annum.

For an application form and further particulars of the post please send a self addressed envelope (9" × 6") to **Artemis Harman, Personnel Office, Sussex House, The University of Sussex, Falmer, Brighton BN1 9RH.** Applicants may discuss the post informally with the Chairman of Physics, Dr D S Betts (Tel: 0273-606755).
AN EQUAL OPPORTUNITY EMPLOYER (8773)A

KING'S COLLEGE LONDON
University of London
BIOMEDICAL SCIENCES DIVISION
LECTURESHIP IN ANATOMY AND HUMAN BIOLOGY

Applications are invited for the post of Lecturer in Anatomy and Human Biology. Applicants should have active research interests in, for example Cell Biology, Developmental Biology, Neurobiology, or some other appropriate field, and be prepared to participate in the teaching of science medical and dental curricula. Experience in human topographical anatomy teaching, while desirable, is not essential.

Salary on scale Lecturer A £9,260–£14,500 pa. + £1,650 London Allowance or Lecturer B £15,105–£19,310 p.a. + London Allowance according to age experience, etc.

For further details, contact **Professor K.E. Webster, Anatomy & Human Biology, King's College London, Strand, London WC2R 2LS (telephone 01-873-2484),** to whom completed applications should be returned by 30th April 1989. (8765)A

POSTDOCTORAL RESEARCH ASSOCIATE

Immediate Opening For Biochemist Or Chemist To Work On Development Of Biosensors Using Antibodies And Antigens Attached To Microelectrodes. Experience With Protein Chemistry And Antibodies Is Desirable, But Not Essential. The Successful Applicant Will Join A Multidisciplinary Team Of Biologists, Biochemists, Surface Chemists And Chemical Engineers Working On Bisensor Technology In The Electronic Design Center At CWRU. Excellent Salary And Fringe Benefits.

Write Or Call: **Prof. James E. Zull, Dept. Of Biology, Case Western Reserve University, Cleveland, OH 44106, 216-368-3572.**

CWRU Is An Equal Opportunity Employer. (NW3451)A

RESEARCH PROGRAM DIRECTORS

ornia Institute of Biological Research R), a non-profit institute, has recently been olished with financial support from Strata- in La Jolla, CA. The Institute is in a beauti- oastal location in close proximity to the Salk tute, Research Institute of Scripps Clinic, ical Biology Institute, La Jolla Cancer earch Center, and the University of Califor- San Diego. CIBR will consist of laboratories research interests in eukaryotic gene ulation, oncogenes, cellular and molecular sformation, control of immune responses, cular biology of differentiation, and mamma- development. CIBR Research Program ctors should demonstrate the ability to in funding from extramural granting agencies. earch Program Directors also will be aided significant core supplementation and may e as consultants to Stratagene with the ortunity to engage in collaborative projects. stigators with appropriate research inter- are encouraged to apply.

d curriculum vitae to: **Personnel, Califor- Institute of Biological Research, 99 North Torrey Pines Road, La Jolla, 92037.** (NW3487)A

SCIENTISTS
CARDIOVASCULAR RESEARCH

COR Therapeutics, Inc. is a recently established and rapidly growing biotechnology company located in the San Francisco Bay Area. We specialize in the development of novel therapeutics for cardiovascular disease. We are actively recruiting a few highly qualified scientists with proven track records to join our Research Department. These scientists will join a research effort directed at the study of molecular mechanisms underlying various aspects of cardiovascular disease. The primary focus will be on growth factors and growth factor receptors, and receptors mediating vascular cell interactions.

Molecular Biology

Expression of native, chimeric and mutant proteins. Experience with mammalian cell expression preferred. Objectives will be to use a molecular approach to understand protein function and to develop the use of expressed proteins as novel pharmaceutical agents.

Protein Biochemistry

Identification, isolation and characterization of structural and functional domains of ligands and receptors. Objectives will be to characterize structure-function relationships of these proteins and to use this information to develop receptor antagonists.

COR Therapeutics, Inc. offers competitive salaries, benefits, and attractive equity positions to its employees combined with the challenge and opportunity to make a significant contribution to this new organization. To apply, please send *curriculum vitae* to COR Therapeutics, Inc., 256 E. Grand Avenue, Suite 80, South San Francisco, CA 94080, ATTN: Human Resources. EOE. (NW3476)A

COR Therapeutics, Inc.

University of York
DEPARTMENT OF BIOLOGY

Postdoctoral Research Fellow

pplications are invited for the above AFRC-funded post, available nmediately for a period of three years. The project involves patch amp recording of channel activity in endomembranes and will focus n the role of calcium channels in signal transduction. Previous xperience with patch clamp would be advantageous, though train- ng in this regard can be provided. The appointee will join a well- quipped laboratory active in various aspects of stimulus-response oupling in plant cells.

tarting salary in the range £9,865–£11,680 per annum, with USS.

wo copies of applications, with full curriculum vitae and naming two eferees, should be sent by Tuesday 14 April 1989 to **Personnel Office, niversity of York, Heslington, YORK YO1 5DD,** from whom further articulars are available. Please quote advertisement reference number /6283. Informal enquiries to Dr D Sanders (tel: 0904 432825)

(8768)A

BIOGROWTH, INC.

ioGrowth, Inc., a biotechnology company located in the an Francisco Bay area, has an immediate opening for OLECULAR BIOLOGISTs to aid in the development of novel uman therapeutic agents. A Ph.D. is required in addition to t least two years postdoctoral experience, with particular mphasis in both cDNA cloning and the expression of genes bacteria or mammalian cells.

ioGrowth offers competitive salaries, benefits, and the pportunity to make a major contribution at a newly develop- ng company. Please send résumé and three references to

BioGrowth, Inc.
3065 Atlas Road, Suite 117, Dept. MB-1
Richmond, CA 94806

(NW3488)A

UNIVERSITY OF SUSSEX

Schools of Chemistry and Molecular and Biological Sciences

Multi-disciplinary Research:

Molecular Recognition Studies of Molybdenum Enzymes

Positions are available for *THREE* individuals, for up to three years, to join a multi-disciplinary team working on the structure and enzymic mechanisms of molybdenum enzymes. The work is supported in part by the SERC Molecular Recognition Initiative. In addition to conventional biochemical techniques, physical methods such as e.p.r., ENDOR and EXAFS spectroscopy will be used, as well as molecular biology, involving site-directed mutagenesis. Applicants should preferably have experience at the postdoctoral level relevant to one or more of the above, or to enzyme purification. However, a less well-qualified applicant, perhaps a new graduate in chemistry or biochemistry, would be considered for one of the positions and would be encouraged to register for a higher degree. In all cases those appointed will be encouraged to train in techniques in which they are not already experienced. Appointments will be made on the RA1A, or RA1B salary scales, as appropriate, at salaries of up to £12,760 or £8,675, respectively.

Applications, including detailed *curriculum vitae* and the names of two referees should be sent as soon as possible to **Dr. R.C. Bray, School of Chemistry and Molecular Sciences, University of Sussex, Brighton, BN1 9QJ, phone (0273) 678398,** from whom further particulars may be obtained.

An Equal Opportunity Employer. (8772)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

MOLECULAR BIOLOGIST

Postdoctoral or postgraduate research assistant required to join project on acetylcholinesterase genes from mosquito vectors of malaria. Includes DNA sequencing and PCR. Some DNA experience preferred. Funded by Wellcome Trust, with salary on 1A or 1B scale. Available from 1st April for one year in first instance. Applications and informal enquiries to **Dr Lucinda Hall, Dept of Medical Microbiology, The London Hospital Medical College, Turner Street, London E1 2AD (Tel: 01-377 7259)** as soon as possible. (8740)A

FACULTY POSITION — X-RAY CRYSTALLOGRAPHY

The Department of Biochemistry, Medical School at the University of Minnesota invites applications for a tenure-track faculty position at the Assistant or Associate Professor level. Candidates with professional distinction in research and writing and demonstrated effectiveness in teaching and advising will be considered for an Associate Professor position. Candidates demonstrating involvement in quality research accepted or published in peer-reviewed journals will be considered for an Assistant Professor position. Applicants should have a Ph.D. degree with expertise in x-ray crystallography and structural analysis of macromolecules. The successful applicant will be part of a group of scientists working in structural biology associated with the William F. Dietrich Chair program. The Structural Biology group complements a core of biophysical scientists in several departments in the Medical School and in the College of Biological Sciences. These departments maintain strong graduate programs in biophysics and molecular biology. The new opening includes ready access to state-of-the-art x-ray diffraction facilities and the Minnesota Supercomputer Institute.

Applications must be postmarked no later than June 1, 1989. Send curriculum vitae, a concise plan for future research, and the names of three references to:

Leonard J. Banaszak, Center for Structural Biology
Department of Biochemistry, Medical School
4-225 Millard Hall, 435 Delaware St. S.E.
University of Minnesota
Minneapolis, MN 55455

The University of Minnesota is an equal opportunity educational employer, and specifically invites and encourages applications from women and minorities. (NW3480)A



STRATAGENE HAS OPPORTUNITIES AVAILABLE IN THE FOLLOWING AREAS:

TECHNICAL SALES REPRESENTATIVES
Positions are available within the Southern California and Midwest Regions. These positions represent a unique opportunity for qualified individuals to participate in the development of a technical sales organization.
Requirements: BS Degree in Molecular Biology or related field. Prior sales experience preferred.

TECHNICAL SERVICE REPRESENTATIVES
Requirements: BS Degree in Biology and 2-3 years' lab experience in Molecular Biology. Previous customer experience preferred. Will be responsible for answering technical inquiries, writing technical literature and telemarketing from our La Jolla, CA office.

PRODUCT DEVELOPMENT ENGINEER
Requirements: BS Degree in Engineering and 3-5 years' experience in instrument design for medium to high volume production. Must be proficient in digital/analog design, packaging and product implementation. Hands on mechanical skills a must.

AUTOMATION PRODUCT DEVELOPMENT ENGINEER
Requirements: Masters or PhD in Biology or Biochemistry. Position involves the interaction of a mechanically oriented biologist with electrical/mechanical engineers to develop automated equipment for the molecular biology laboratory. Qualified individuals should have an interest in optomizing molecular biology procedures and protocols with the prospective of adapting these protocols to automation.

LABORATORY TECHNICIANS
Requirements: BS Degree in Molecular Biology with 2-3 years' practical working experience in a biotech environment.

QUALITY ASSURANCE TECHNICIANS
Requirements: BS Degree in Molecular Biology with a minimum of 2-3 years' work experience in a biotech environment. Must have knowledge of the following: DNA Sequencing, cloning techniques, plasmids, restriction and modifying enzymes, protein purification and DNA electrophoresis.

Stratagene offers a beautiful working environment, competitive salaries and excellent benefits. All qualified candidates forward resume of qualifications to:

Stratagene, Attn: Human Resources Dept.
11099 N. Torrey Pines Rd., La Jolla, CA 92037 (NW3486)A

THE AUSTRALIAN NATIONAL UNIVERSIT

Research School of Earth Sciences

POSTDOCTORAL FELLO
RESEARCH FELLOW
IN ENVIRONMENTAL
GEOCHEMISTRY

Applications are invited from lo temperature goechemists, geo gists or physicists to underta research in accelerator ma spectrometry. Applicants ne not have hands-on experience w AMS, but will be involved expanding the current collabo tive program of ^{36}Cl research ^{129}I and ^{10}Be with possible la development of ^{41}Ca and ^{53}M The successful applicant will ass in developing new areas research using the 14MV Pelletr in the Department of Nucl Physics, ANU.

The Environmental Geochen try Group investigates ne surface geochemical processes a paelaeoenvironments of the A tralian continent and its surroi ding margins. Current proje include stable-isotope, radioi tope and trace-element studies inland lake sediments, mari sediments and the weathei mantle or regolith.

Prospective applicants shoi write for further particulars q ting the reference number, bef submitting applications.

Closing date: *14 April 19* Ref: ES 3.3.1

SALARY: Research Fell A$30,737-A$40,100 p.a.; P doctoral Fellow Grade I (fi point); A$26,617-A$30,360 APPOINTMENT: Resea Fellow up to three years, poss lity of extension to five years; P doctoral Fellow normally years, possibility of extension three years. Applications sho be submitted in duplicate to Registrar, The Australian Nati al University, GPO Box 4, C berra ACT 2601, Australia, q ting reference number and in ding curriculum vitae, list of pu cations and names of at least th referees. The University reser the right not to make an appo ment or to appoint by invitatio any time. Please obtain furt particulars before applying fr the Registrar, or from Appo ments (36120), Association Commonwealth Universities, Gordon Square, London WC 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER
(W5969)

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nature
— the professionals choice

AUSTRALIA
CSIRO DIVISION OF SOILS
RESEARCH OFFICER

We are seeking an enthusiastic Research Fellow with a research background in the fields of Fluid Mechanics/Hydraulics/Sedimentology/Soil Erosion to work in our well equipped Canberra laboratory on soil erosion by water. Particular attention will be given to the interaction between rain, shallow surface flow, vegetation and surface roughness on the erosion process.

The appointment is for a term of 5 years with a salary range of A$31,003 p.a. to A$45,177 p.a.

The successful applicant will have a PhD or equivalent qualification in fluid mechanics, hydraulics, soil erosion processes or related field; appropriate skills in mathematics and numerical modelling; good communication and collaborative skills. Practical experience with soil physical or hydrological experimental procedures in the laboratory and in the field is desirable.

Prospective applicants are invited to discuss the position with Dr. J. Williams phone (6177) 71 9511 or Dr. P. Walker phone (6162) 46 5318.

More information can be received by contacting Ms. G. Mortan phone (6162) 46 5322 FAX (6162) 47 5883, or Mrs. J. Clark phone (618) 274 9206 FAX (618) 338 1636.

Please send full C.V. including names of at least 2 referees and ref: AA301, to:

The Chief
CSIRO Division of Soils
G.P.O. Box 639
Canberra, A.C.T. 2601
Australia. (W5983)A

University of York
Department of Biology
Cancer Research Unit
Research Assistant

The above three year position is available for a graduate, or those who are about to graduate in the biological sciences, to investigate the role of oncogene activation and chromosome alterations in human colon cancer. The research will involve cell culture and molecular biology techniques.

Candidates should have, or expect to obtain, at least an Upper Second Class honours degree. The successful applicant could be eligible to register for a higher degree.

Salary will be on the range 1b scale for research and analogous staff, £8,675 – £11,680 per annum.

Informal enquiries can be made to Dr Colin Garner on 0904 432900.

Four copies of applications with full curriculum vitae and naming two referees, should be sent by Friday 7 April 1989 to the Personnel Office, University of York, Heslington, YORK, YO1 5DD, from whom further partiuclars are available. Please quote advertisement reference number 7/6282. There are no printed application forms. (8725)A

POSTDOCTORAL POSITIONS

Postdoctoral positions are available in cell, molecular and structural biology of membrane (D.W. Hamilton) and cytoskeletal systems (R.W. Linck). Membrane projects include: biogenesis during spermatogenesis, glycosylation, formation of the acrosomal, head and flagellar membranes, subsequent membrane maturation within the epididymis, and the function of membrane proteins. Cytoskeleton projects include: molecular structure and assembly of microtubules, gene structure and expression of microtubule proteins in development, and in vivo function of microtubules in cilia/flagella, centrioles/centrosomes and mitosis/meiosis. Experience is required in preferably more than one of the following: cytology, protein chemistry, immunochemistry, EM, diffraction methods, and/or recombinant DNA methods. Send curriculum vitae and three letters of reference to: **Dr. D.W. Hamilton or Dr. R.W. Linck, Department of Cell Biology and Neuroanatomy, University of Minnesota, 4-135 Jackson Hall, 321 Church Street, S.E., Minneapolis, MN 55455.** (NW3218)A

SEARCH RE-OPENED
TENURE-TRACK FACULTY POSITION
IN PALEOCEANOGRAPHY

The Institute of Marine Science, University of Alaska Fairbanks, has re-opened its search for a tenure-track faculty member in paleoceanography. The appointment will be with the Institute of Marine Science in the recently organized School of Fisheries and Ocean Sciences and involves research, graduate-level instruction, and direction of graduate student research. The Institute of Marine Science has a faculty of 24 and about 40 graduate students at the M.S. and Ph.D. level. Institute faculty are involved in a variety of research programs emphasizing arctic and sub-arctic seas and are actively planning university-wide programs addressing global change.

We seek a colleague committed to collaborative work in paleoceanography in a broad sense and welcome applicants bringing modern approaches from geology, physics, chemistry or biology. The appointee must hold a Ph.D. and will be responsible for developing an active research program in paleoceanography. We anticipate filling this position at the assistant professor level, but will consider a more senior appointment in exceptional cases.

Applicants should send (by April 30, 1989) a statement of research interests, a curriculum vitae, and the names and addresses of three references to:

William S. Reeburgh
Chair, Paleoceanographer Search Committee
C/O Director's Office
Institute of Marine Science
University of Alaska Fairbanks
Fairbanks, Alaska 99775-1080

Applications of finalists may be subject to public disclosure. Persons hired must comply with the new employment provisions of the Federal Immigration Reporting Act. The University of Alaska is an Equal Opportunity/Affirmative Action Employer, educational institution, and could become a year-round paradise with a little global warming. (NW3470)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF ZOOLOGY
CANCER RESEARCH CAMPAIGN
DNA REPAIR RESEARCH GROUP
RESEARCH ASSISTANT

A graduate research assistant is required to work on biochemical aspects of DNA repair. This post is available from 1 April 1989 and, for a suitable person, will extend for five years from 1 January 1990.

We are looking for an individual who can take responsibility for running and developing assays for DNA repair within a small team of scientists. Applicants should have a good first degree plus some experience in protein and/or nucleic acid biochemistry. A background in microbiology or molecular biology would also be useful.

Starting salary will depend on age and experience and will be in the University of Cambridge Research Assistant Scale (£9,260 to £12,760).

Applications in writing, together with c.v. and names and addresses of two scientific referees should be sent to **Mr J R Andrews, Departmental Secretary, Department of Zoology, Downing Street, Cambridge CB2 3EJ.**

Further information can be obtained informally by contacting Dr R T Johson (0223) 336672. (8703)A

POSTDOCTORAL RESEARCH ASSOCIATE

needed to join project on the structural characterization of glycoproteins (primarily the AIDS virus coat protein HIV gp120); emphasis is on the structures of the carbohydrates and their sites of attachment to the protein. Ph.D. degree required in chemistry/biochemistry; expertise in the characterization of carbohydrates using chemical techniques, mass spectrometry, and NMR spectroscopy is desirable. Salary $18,000-24,000 depending on experience. Send applications, which will be evaluated beginning April 15, 1989, to **Dr. Alan Darvill or Dr. Herman van Halbeek, Complex Carbohydrate Research Center, P.O. Box 5677, Russell Labs, Athens, GA 30613.** An AA/EEO institution. (NW3482)A

UNIVERSITY OF CAMBRIDGE

University offices: Vacancies under the New Academic Appointments Scheme

Applications are invited for the following University offices under the U.G.C. New Academic Appointments Scheme, which is designed to provide for the appointment of more younger staff to permanent academic positions. Appointments will be made from 1 October 1989 or as soon as possible thereafter, subject to confirmation by the U.G.C. of the provision of the necessary funds. The Appointments Committees concerned hope soon to be in a position to make these appointments.

University Lectureships, University Assistant Lectureships and Assistant Directorships of Research

Tenure

University Lecturers and University Assistant Lecturers: Appointments will be for three years; with the possibility, for a University Lecturer, of reappointment to the retiring age or, for a University Assistant Lecturer, of reappointment for two years and of promotion to a University Lectureship.

Assistant Directors of Research: Appointments will be for up to five years, with the possibility of reappointment for periods not exceeding five years at a time. The General Board, on the recommendation of the Appointments Committee concerned, are empowered to authorise the reappointment to the retiring age of an Assistant Director of Research who has held that office continuously for not less than five years.

Stipends

University Lecturers: £13,365 a year, rising by eleven annual increments to £20,615. There is no grade of Senior Lecturer.

Assistant Directors of Research: £13,365 a year, rising by nine annual increments to £19,310.

University Assistant Lecturers: £10,460 a year, rising by seven annual increments to £14,500.

University Assistant Lecturer in the Department of Geography. It is intended to appoint a Physical Geographer with particular interests in applying scientific approaches to the investigation of contemporary environmental problems.

Further information may be obtained from the Secretary of the Appointments Committee for the Faculty of Earth Sciences and Geography, Department of Geography, Downing Place, Cambridge, CB2 3EN, to who applications (ten copies) including a *curriculum vitae* and the names of three referees, should be sent so as to reach him not later than 5 April 1989.

Assistant Director of Research in the Institute of Astronomy. The main duties of the person appointed will be research and the supervision of graduate students. Applications are welcome from astronomers whose research relates to any part of the Institute's programme. Closing date 28 April 1989.

Further information about this office may be obtained from the Secretary of the Appointments Committee for the Faculty of Physics and Chemistry, Institute of Astronomy, Madingley Road, Cambridge, CB3 0HA, to whom applications (ten copies), including a *curriculum vitae* and the names of three referees, should be sent so as to reach him not later than the closing date given.

University Lecturer or University Assistant Lecturer in the Department of Zoology. Preference will be given to a person working in the area of *either* population biology, particularly with an interest in theoretical or empirical work on animal populations, *or* comparative physiology, particularly with an interest in animal movement and related subjects.

Further information may be obtained from the Secretary of the Appointments Committee for the Faculty of Biology 'A', Department of Zoology, Downing Street, Cambridge, CB2 3EJ, (Tel: 0223 336621) to whom applications (twelve copies) including a *curriculum vitae*, list of publications and the names of not more than three referees, should be sent so as to reach him not later than 19 May 1989.

University Lecturer or University Assistant Lecturer in the Department of Experimental Psychology. An appointment will be made in the general area of cognitive psychology, neuropsychology or developmental psychology, with preference given to someone working on computational modelling of cognitive processes or associative or neural networks. Closing date 17 April 1989.

University Lecturer or University Assistant Lecturer in Parasitology in the Department of Pathology. Candidates should have several years' post-doctoral experience in parasitology and in the application of molecular, biochemical or immunological techniques to the investigation of the developmental biology of parasites and of host/parasite relationships. The successful candidate will be expected to teach natural science, medical and veterinary students at undergraduate and postgraduate levels. Closing date 8 April 1989.

University Lecturer or University Assistant Lecturer in the Department of Pharmacology. The Department is about to move to new, custom-built premises and the lecturer will be provided with a well-serviced laboratory and office space. The successful applicant must be committed to a study of the mechanisms of drug action, the discovery of new drug targets and mechanisms and be competent at giving undergraduate courses. Furthermore the lecturer will be able to attract research support. There is no restriction of field for applicants, but preference will be given to those with proven expertise in the new electrophysiological methods or with abilities in molecular and/or cellular genetics. The successful applicant is expected to be able to relate his/her research to the activities of at least one of the major research groups in the department, such as receptors and ion channels, membrane transport especially in epithelia, or transduction and signalling mechanisms. Closing date 8 April 1989.

Further information about the duties and conditions of appointment of any of these three offices and an application form may be obtained from Dr D. Franks, Secretary of the Appointments Committee, 19 Trumpington Street, Cambridge, CB2 1QA, to whom applications (ten copies) including a *curriculum vitae*, list of publications and the names of not more than three referees, should be sent so as to reach him not later than the closing date given.

University Lecturer in Equine Medicine in the Department of Clinical Veterinary Medicine. Applicants should have a veterinary degree registrable with the Royal College of Veterinary Surgeons and some experience of the practice of equine veterinary medicine. Possession of a higher degree and/or an appropriate Diploma of the RCVS will be an advantage. The successful applicant will be expected to develop teaching in the field of equine medicine and a research programme in an appropriate area of that field.

University Lecturer in Clinical Pathology/Small Animal Medicine in the Department of Clinical Veterinary Medicine. Applicants should have a veterinary degree registrable with the Royal College of Veterinary Surgeons and some experience of the practice of small animal medicine, together with experience of clinical pathology, including clinical haematology and clinical biochemistry. Possession of a higher degree and/or an appropriate Diploma of the RCVS will be an advantage. The successful candidate will be expected to develop teaching in the fields of clinical pathology and small animal medicine and the related research programmes in an appropriate area of these fields.
Further information about the duties and conditions of appointment of these two offices may be obtained from the Secretary of the Appointments Committee for the Department of Clinical Veterinary Medicine, Madingley Road, Cambridge, CB3 0ES, to whom applications (twelve copies), including a *curriculum vitae*, list of publications, and the names of not more than three referees, should be sent so as to reach him not later than 17 April 1989. (8777)A

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ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

Anatomical Society Research Studentships

Applications are invited from Departments of Anatomical Sciences in the UK and Ireland for Research Studentships tenable in the Anatomical Sciences for a period up to three years. Students nominated by Departments must be graduates of British or Irish Universities and will be expected to register for a higher degree. The stipend will be commensurate with basic Research Council rates.

Anatomical Society Senior Visiting Fellowship

Applications are invited from Overseas Scientists for *one* Fellowship tenable for a period of less than a year in a Department of Anatomical Sciences in the UK or Ireland. Applicants should be of post-doctoral or comparable status and must have arranged sponsorship in the Department in which they intend to work. Some assistance towards travel and subsistence will be provided.

Further particulars should be obtained from the Acting Secretary, Dr Ian Whitmore, Department of Cell and Structural Biology, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, to whom applications should be sent by 28 April 1989.

(8606)A

BOTANIST

The National Tropical Botanical Garden (formerly Pacific Tropical Botanical Garden), the only national, privately supported, tropical botanical garden chartered by the United States Congress, is seeking to fill a full-time position available immediately. A special Hawaiian Islands Plant Conservation Center is being created at the Garden's headquarters on Knual with funds made available by the John D. and Catherine T. MacArthur Foundation. This will be a cooperative effort to establish a network of botanical gardens and research institutions in Hawaii. The successful candidate will coordinate efforts to establish this new center for the study and conservation of Hawaii's rare and endangered vascular plants and also develop and implement strategies for preserving these plants. A Ph.D. in a relevant field or equivalent experience is required. Experience working with Hawaiian plants is preferred. The ability to communicate well in writing and speaking and a knowledge of computers are advantageous. Salary is commensurate with experience. Initially this program will be supported by a 3-year grant from the MacArthur Foundation. Closing date for applications is March 31 1989 or until a suitable candidate is found.

Applicants should submit their curriculum vitae, description of research interests, and provide the names and addresses of three references to: **Dr. William L. Theobald, Director, National Tropical Botanical Garden, P.O. Box 340, Lawai, Kauai, HI 96765.**

The National Tropical Botanical Gardens is an Equal Opportunity Employer. (NW3469)A

POSTGRADUATE RESEARCH DEVELOPMENTAL NEUROBIOLOGIST

A position exists for research on the development of the nervous system of the leech. Current projects include the use of injection cell lineage tracers alone and in combination with other techniques to study the role of lineage in determining cell fate and factors governing differential cell migration in histogenesis. Expertise with techniques of microinjection and familiarity with leech embryology valuable; ideal candidate should be conversant with and able to develop new approaches from the fields of neurobiology, developmental biology, immunology and biochemistry. Salary range: 23,146-34,788 depending on qualifications. Send resume, description of research experience and bibliography by April 15, 1989 to **Dr. G.S. Stent, Department of Molecular Biology, University of California, Berkeley, CA 94720.**

The University of California is an Equal Opportunity/ Affirmative Action Employer. (NW3485)A

Sunderland Polytechnic operates a wide range of courses at degree and postgraduate level, and has a vigorous and successful record of consultancy and research, both of which are encouraged and promoted positively. With the advent of Corporate Status the Polytechnic is entering a new and challenging phase of its development. To maintain and extend the programme of work, applicants are invited for the following posts:

FACULTY OF SCIENCE
SCHOOL OF PHARMACEUTICAL AND CHEMICAL SCIENCES

Lecturer/Senior Lecturer in Formulation Science and Pharmaceutical Microbiology

Applicants are invited for **two** posts. The first post is responsible for teaching formulation science within the suite of courses in pharmaceutical sciences to honours degree level (Pharmacy, Chemical and Pharmaceutical Science) and Post Graduate level (Post Graduate Diploma in Quality Assurance) and to teach aspects of Law and its Administration as applied to the Practice of Pharmacy and Pharmaceutical Sciences.

The second post is responsible for teaching pharmaceutical microbiology for the same suite of courses and to Pharmacology students to honours degree level.

In both cases experience in research or industry or consultancy, in these areas will be an advantage. You will be required to participate in the research programme of the School.

Lecturer/Senior Lecturer in Analytical Biochemistry

You will be responsible for teaching analytical biochemistry and clinical chemistry to honours degree level and for co-ordinating developments in research, teaching and consultancy in these areas.

Salary Scales:
Lecturer: £8,451 to £15,369 per annum
Senior lecturer: £15,369 to £18,549 per annum

Application forms and further particulars can be obtained from Personnel Services, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland SR2 7EE or telephone (091) 5152429.

Closing date: 17th April 1989.

(8754)A

SUNDERLAND POLYTECHNIC

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2A WESTBRIDGE ROAD, LONDON SW11 3PW TEL: 01-223 3497

(8495)A

ASSISTANT/ASSOCIATE PROFESSOR OF TROPICAL PUBLIC HEALTH

The Department of Tropical Public Health at the Harvard School of Public Health is searching for an Assistant/Associate Professor to work on the biological aspects of malaria. The candidate should have a record of productivity on the study of malaria. The individual would join an active department employing a multidisciplinary approach to the study of a number of different protozoa and helminths causing disease in humans and would be expected to develop an independent research and teaching program. As a faculty member of this department, the person should be willing to become involved in projects on malaria research overseas.

Applicants should send curriculum vitae including list of publications and reprints of representative recent publications to:

***ad hoc* Committee on Malaria**
Department of Tropical Public Health
Harvard School of Public Health
665 Huntington Avenue
Boston, MA 02115

The Harvard School of Public Health is an equal opportunity employer and applications from women and minority groups are encouraged. (NW3481)A

Endowed Chair in Agricultural Biotechnology

The Oklahoma Agricultural Experiment Station has established an endowed chair to complement existing interdisciplinary research programs in Arthropod-Host and/or Phytopathogen-Host Interactions. The department of academic appointment will be dependent on the research interests and qualifications of the candidate. Qualifications include an earned doctoral degree in an appropriate area of science, six years of research experience, an excellent record of publications, the ability to successfully obtain outside support, and the ability to work cooperatively with other scientists. Startup equipment funding and continuing research support is available from a $1 million endowment. The biotechnology program is support by the new $45 million Noble Research Center for Agriculture and Renewable Natural Resources. Applications should include a letter indicating qualifications and a general description of research achievements and goals, a complete curriculum vita and the name and address of at least five references.

Applications will be accepted until a suitable candidate is identified. Nominations or applications should be sent to: **Dr. Tom Haan, 124 Agricultural Hall, Oklahoma State University, Stillwater, OK 74078.**

Oklahoma State University is an Affirmative Action-Equal Opportunity Employer. (NW3454A)

THE UNIVERSITY OF CALIFORNIA, SAN DIEGO

is seeking a Ph.D. applicant for a Postgraduate Researcher (PGR). Candidates should have experience in molecular biology and retrovirology or herpesvirology to conduct research in AIDS pathogenesis and the role of viral cofactors. Position available immediately for at least 2 years. Salary range based on University of California policy. Interested individuals should send curriculum vitae with a list of three references no later than June 30, 1989 to: **Stephen A. Spector, M.D., Associate Professor, Department of Pediatrics, UCSD Medical Center 225 Dickinson Street (H–814–H), San Diego, CA 92103.**

Equal Opportunity/Affirmative Action Employer. (NW3434)A

RESEARCH ASSOCIATE OR POSTDOCTORAL

position available 1989 to study biochemistry or molecular biology of protein degredation in the eye lens, particularly as it is affected by aging cataracts or nutrition. *Must be able to prove eligibility to work in the United States.* A list of our recent papers is available. Please send curriculum vitae and names of three references to:

Dr. Allen Taylor
USDA Human Nutrition Research Center
Tufts University
711 Washington Street Boston, MA 02111

An Affirmative Action/Equal Opportunity Employer. (NW3447)A

NATIONAL HEART AND LUNG INSTITUTE
University of London
Dovehouse Street, London SW3 6LY

An experienced histologist is required to join a newly formed pharmacology group in the Department of Cardiothoracic Surgery at the National Heart and Lung Institute and the Transplant Unit at Harefield Hospital. The main project involves the identification and localisation of receptors for vasoactive peptides in human cardiac tissue using in vitro autoradiography. This is to be run in parallel with functional studies measuring changes which occur in certain coronary conditions such as ischaemic heart disease. We are seeking a person with sound histological skills. Experience in any of the following areas would be advantageous — autoradiography, immunocytochemistry, photography, in situ hybridisation. The successful candidate would play an important role in our ongoing research commitment and would be encouraged to become involved in other specialised areas such as image analysis.

Applications (in the form of CV and names and addresses of two referees) should be addressed to **Professor M. Yacoub, The National Heart and Lung Institute, Dovehouse Street, London SW3 6LY.** Informal enquiries may be made to **Dr. M. Dashwood, Pharmacology Department, Harefield Hospital (0895-82-3737).** (8745)A

INSTITUTE OF ZOOLOGY
ZOOLOGICAL SOCIETY OF LONDON

DEVELOPMENTAL BIOLOGIST

ZOOLOGICAL SOCIETY OF LONDON

Required to work on systems for pre-implantation identification of genetic characteristics in primate embryos. The position provides an opportunity for the study of the embryology of the marmoset money, utilizing the Institute's extensive facilities for research on the marmoset, as well as for collaboration with molecular geneticists on the development and use of gene probes. The work will include investigations on gene expression on the developing embryo. The laboratories are equipped with appropriate embryo micromanipulation, culture and freezing facilities, and applicants should have experience in these techniques, as well as in surgical procedures; some knowledge of molecular biology would also be an advantage.

The position is for 5 years (3 years in the first instance), funded by a joint MRC/AFRC Programme Grant. Salary, aligned to University Lecturer scale, in the range £13,869–£19,116 plus London Weighting. Further details can be obtained from **Professor A P F Flint, Director, Institute of Zoology, Regent's Park, London NW1 4RY (tel: 01-722 3333),** to whom written applications, including names of three referees, full CV and publication list, should be sent by 13th April 1989. (8729)A

FACULTY IN NEUROPHARMACOLOGY

Tenure-track positions available in the Department of Pharmacology Louisiana State University Medical Center-Shreveport for accomplished researchers to develop independent research programs, and to participate in medical and graduate teaching.

Specialization in receptor mechanisms, neuropeptide, behavioral pharmacology, or nervous-immune system interactions preferred. Send CV, publications list, summary of completed research and future plans, and the names of three referees to **Dr. Adrian J. Dunn, Department of Pharmacology, LSUMC-S, P.O. Box 33932, Shreveport, LA 71130.** *EOE/AA* (NW3471)A

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National Cancer Institute–Frederick Cancer Research Facility
BIONETICS RESEARCH, INC.–BASIC RESEARCH PROGRAM
POSTDOCTORAL FELLOWSHIPS

BRI–Basic Research Program Postdoctoral Fellowships are awarded for 1–2 years with stipends ranging from $24,000 to $30,000/year. The senior staff members and their research interests are

George Vande Woude: molecular basis of neoplastic transformation

Stephen Hughes: structure and function of HIV reverse transcriptase and integration protein; expression of cytoskeletal genes; retroviral vectors; transgenic birds

George Pavlakis: eukaryotic gene regulation; molecular mechanisms of cell transformation; molecular biology of HIV and pathogenesis of AIDS

Peter Johnson: mammalian transcription factors; molecular basis of tissue-specific gene expression

Stephen Oroszlan: immunochemistry and protein chemistry of retroviruses; structure and function of retroviral gene products; proteases

Alan Rein: retroviral genetics; functional analysis of retroviral genes using natural and synthetic mutants

Nancy Rice: molecular biology of retroviruses; oncogene expression

William Lijinsky: environmental carcinogenesis; mechanisms of carcinogenesis by nitrosamines and related compounds

Anthony Dipple: carcinogen–DNA interactions; polycyclic aromatic hydrocarbon carcinogenesis and mutagenesis

Robert Moschel: chemical synthesis of carcinogen–modified DNA; physical chemistry of carcinogen–DNA interactions; DNA adduct–induced mutagenesis in bacteria and mammalian cells

Christopher Michejda: biochemistry of carcinogen activation; DNA alkylation; chemotherapeutic agents

Jeffrey Strathern: cell type regulation; genome rearrangement, recombination, and DNA repair in yeast

David Garfinkel: molecular biology of the yeast retrotransposon Ty1; genome rearrangement and evolution

Amar Klar: gene regulation and homothallic mating–type switching in fission and budding yeast

Stuart Austin: chromosome stability in bacteria: regulation of plasmid replication and distribution of copies to daughter cells

Richard Fishel: molecular mechanisms and biochemistry of genetic recombination

Donald Court: regulation of gene expression by transcription initiation, transcription termination, and RNA processing

Neal Copeland: molecular genetics of murine leukemogenesis; developmental genetics; transposable elements and retroviruses

Nancy Jenkins: insertional mutagenesis by retroviral DNAs; transgenic mice; molecular biology of mouse development

Luis Parada: use of pluripotential stem cells to study molecular biology of development and the role of oncogenes; *in situ* hybridizations

Peter Donovan: development of the germ line; germ line mutations; cell matrix interactions

Alexander Wlodawer: structure of enzymes and oncogene products studied by X-ray diffraction

Irene Weber: crystallographic investigation of proteins, DNA, and protein–nucleic acid interactions

J. Ronald Rubin: crystallographic investigations of ligand–macromolecule interactions and drug–nucleic acid interactions

Send a curriculum vitae and the names of three references to Dr. Maurice L. Guss, BRI–Basic Research Program, NCI–Frederick Cancer Research Facility, P.O. Box B (Rm 48), Frederick, Maryland 21701.

An equal opportunity/affirmative action employer M/F/H/V

(NW3340)E

RESEARCH FELLOWSHIPS IN GENETIC RECOMBINATION
£13,300-£16,000

The Imperial Cancer Research Fund is one of the largest independent cancer research institutes in Europe, employing approximately 400 scientists and clinicians. It has a wide ranging programme in fundamental, applied, and clinical cancer research.

Applications are invited for two postdoctoral positions:

(1) The mechanism of homologous pairing and strand exchange by the E. coli RecA protein.

(2) Resolution of recombination intermediates (Holliday junctions) in vitro.

Candidates for both positions should have experience in the techniques of molecular biology. Additional experience in cloning and gene manipulation would be advantageous for project (2). The fellowships are available at the ICRF's Clare Hall Laboratories situated 10 miles north of London and are tebable for a period of three years. Further information can be obtained from Dr S. West 0797-44444 ext 316.

Applications comprising a full curriculum vitae and the names and addresses of three referees should be sent to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX quoting reference 80/R.

Smoking is actively discouraged.

I M P E R I A L
CANCER RESEARCH FUND (8775)E

UNIVERSITY OF ZURICH, SWITZERLAND

POSTDOCTORAL FELLOWSHIP
in
MOLECULAR BIOLOGY

An enthusiastic post-doctoral scientist preferably with experience in the areas of cloning, gene transfer or site-directed mutagenesis, is required to join a multi-disciplinary team actively involved in research on chemically gated ion channels in the central nervous system (see Proc. Nat. Acad. Sci. 85, 7815, 1988).

The current project aims to study the role fo the GABA-receptor in regulating anxiety, vigilance and epileptic seizures. Investigation will focus on the structural heterogeneity, functional divergence and regulation of gene expression of this receptor family in normal and diseased brain.

Applicants for the three-year post, now available, should send CV, list of publications and the names of three referees to

Prof. H. Mohler,
Neuroscience Dept.,
Gloriastr. 32,
CH 8006 Zurich,
Switzerland.

(W5953)E

THE MORRISON MEMORIAL POSTDOCTORAL FELLOWSHIP

The Department of Biochemistry, St. Jude Children's Research Hospital, is enlarging its research efforts in the area of the molecular and cellular biology of signal transduction. As part of this expansion, the Board of Governors has established a memorial fellowship in honor of Dr. Martin Morrison, the founding chairman of the Department. The research programs within the Department are well equipped and offer recent Ph.D. or M.D. graduates the opportunity to participate in energetic, multidisciplinary groups dealing with a range of basic and clinically oriented research. Current research programs within the Department include:

W.Y. Cheung: Regulatory properties and biological functions of calmodulin; Ca^{2+} regulated enzymes.

J. Cleveland: Growth factor regulation of gene transcription.

V.A. Fried: The ubiquitin system and post-translational regulatory pathways.

J.N. Ihle: Mechanisms of hematopoietic stem cell growth regulation, differentiation and transformation.

S. Jackowski: Regulation of phospholipid metabolism and the production of lipid-derived second messengers.

C. Rock: Hormone and growth factor stimulated phospholipase *C*.

E. Thomas: Antimicrobial and antitumor mechanisms of leukocytes.

Applicants should submit a brief statement of their research interests, curriculum vitae, reprints and names of three references to:

The Morrison Postdoctoral Fellowship
Department of Biochemistry
St. Jude Children's Research Hospital
332 N. Lauderdale
Memphis, TN 38101

An Equal Opportunity/Affirmative Action Employer

(NW3401)E

WEIZMANN FELLOWSHIP

The Division of Biology, California Institute of Technology invites applications for Prize Postdoctoral Fellowships for Scientific Research. The competitively awarded Fellowships will be for one year initially; Fellows may compete for a second award. The annual stipend is $32,000 to the awardee plus small research and travel allowances. Applicants must be citizens of the U.S. or Israel. Work may be associated with any faculty member of the Division.

Applications, including curriculum vitae, list of publications, and research plan received by April 15, 1989 will be considered for the 1989–90 academic year. Address inquiries to **Dr. Norman Davidson, Interim Chairman, Division of Biology, Mail Code 156–29, California Institute of Technology, Pasadena, CA 91125**

The Institute is an Equal Opportunity, Affirmative Action Employer and welcomes applications from women and members of minority groups.

(NW3472)A

BANTRELL FELLOWSHIP

The Division of Biology, California Institute of Technology invites applications for Prize Postdoctoral Fellowships for Scientific Research. The competitively awarded Fellowships will be for one year initially; Fellows may compete for a second award. The annual stipend is $32,000 to the awardee plus small research and travel allowances. Applicants must be citizens of the U.S. or Israel. Work may be associated with any faculty member of the Division.

Applications, including curriculum vitae, list of publications, and research plan received by April 15, 1989 will be considered for the 1989–90 academic year. Address inquiries to **Dr. Norman Davidson, Interim Chairman, Division of Biology, Mail Code 156–29, California Institute of Technology, Pasadena, CA 91125.**

The Institute is an Equal Opportunity, Affirmative Action Employer and welcomes applications from women and members of minority groups.

(NW3473)E

NATIONAL INSTITUTES OF HEALTH
POST DOCTORAL FELLOWSHIP
TRANSGENIC MICE

Experience in molecular biology to work in laboratory using transgenic mice to study the pathogenesis of viruses (e.g., HIV and HSV) and autoimmune diseases. Initial appointment will be for two years, with renewals on a yearly basis. Starting salary range, depending on experience, is $24,000 to $40,000 per year. The position is available immediately. Send Curriculum Vitae and names of references to **Dr. Lois Salzman, Deputy Director, Intramural Research Program, National Institute of Dental Research, National Institutes of Health, 9000 Rockville Pike, Building 30, Room 121, Bethesda, Maryland 20892.** (NW3428)E

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BIOSEM
Groupe LIMAGRAIN

Molecular and Cellular Laboratory Located on the University campus of Clermont-Ferrand, **France,**

Post Doctoral Position

Available immediatly - Duration 2 years.

Application invited for a post doctoral fellowship in plant molecular biology - Applicants must have also a strong experience in plant tissue culture.

Send curriculum vitae and references as soon as possible to :

Groupe LIMAGRAIN
Departement du Personnel
BP 1 - Chappes -
63720 ENNEZAT - France.

(W5967)E

UNIVERSITY OF CAMBRIDGE
Department of Zoology
POSTDOCTORAL FELLOWSHIP IN NEUROBIOLOGY

A postdoctoral position is available to study local neural networks in insects with Professor M Burrows. The position, which is funded by an SERC rolling programme for at least 3 years, can start within 6 months of June 1989. The post offers an opportunity to join an active interdisciplinary research group. Experience in any modern neurobiological technique is essential.

Salary up to £14,500 pa depending on age and experience.

Please send applications, including the names of two referees, to **Professor M Burrows, Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, England.**

(8705)E

University of Reading
School of Plant Sciences
Department of Horticulture

Applications are invited for a 3 year, AFRC-funded Postdoctoral Research Fellowship on the signal transduction pathway linking changes in intracellular Ca^{2+} to cellular growth responses. Applicants should have experience in protein biochemistry; immunology would be an advantage. Salary scale £9,865 to £11,070 p.a. Informal enquiries to Dr N. H. Battey, telephone (0734) 318071. Apply for Application Form and further particulars to **Personnel Officer, University of Reading, Whiteknights House, PO Box 217, Reading RG6 2AH, telephone (0734) 318754.** Closing date 17 April 1989. Please quote Ref. R8915.

(8708)E

The Queen's University of Belfast and Trinity College, Dublin

RESEARCH FELLOWSHIPS

New Perspectives in DNA Photochemistry

Applications are invited from suitably qualified postgraduate or postdoctoral candidates (preferably citizens of the EEC) for two fellowships to conduct joint research on new developments in DNA photochemistry, emphasising laser-induced processes and sequence directed photosensitisation. One fellowship will be held at Queen's University (Dr. R.J.H. Davies, Department of Biochemistry) and one at Trinity College (Professors J.M. Kelly and D.J. McConnell, Departments of Chemistry and Genetics). The programme, funded by the EEC, involves collaboration with Professor C. Helene and Dr. Le Doan, Laboratoire de Biophysique, Museum National d'Histoire Naturelle, Paris. Generous travel funds are available to support consultation and secondments between the three laboratories.

The appointments, commencing as soon as possible, will be for one year with the possibility of renewal; salaries according to age, qualifications and experience.

Applicants, quoting ref. 89/S, should submit a curriculum vitae including the names and addresses of two referees to the **Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland** or to **Professor J. M. Kelly, Chemistry Department, Trinity College, Dublin 2, Ireland.** Informal telephone enquiries to Dr. Davies, Belfast (0232)-329241 ext. 2742 or Professor Kelly, Dublin (0001) 772941 ext. 1947. (8770)E

AGRICULTURAL AND FOOD RESEARCH COUNCIL INSTITUTE OF ANIMAL PHSYIOLOGY & GENETICS RESEARCH, BABRAHAM, CAMBRIDGE CB2 4AT

POST DOCTORAL APPOINTMENT IN DEVELOPMENTAL BIOLOGY

Applications are invited for a three year fellowship to work on molecular or cell biological aspects of oogenesis in mammals. The successful candidate will join a group in the Department of Molecular Embryology who are studying cell interactions, nuclear-cytoplasmic relationships and gene expression in growing oocytes. The work involves cell biology, molecular biology, nuclear transfer and transgenic techniques. The precise area of research will take into account the interests of the successful candidate.

Applicants should have practical experience in molecular biology or a desire to learn these skills. No prior experience in working on gametes is necessary.

The appointment will be for three years in the grade of Higher Scientific Officer. Salary in the range of £10,026–£13,460 according to qualifications and experience. 25 days annual leave and 10.5 public holidays. Non-contributory superannuation scheme. Application forms available from the Personnel Office quoting Ref: TAP35/HSO by 31 March 1989. (8774)E

Please mention

nature

when replying to these advertisements

RESEARCH POSTDOCTORAL FELLOWSHIP

Biochemical and molecular mechanisms of hormone (angiotensin) actions in heart. Receptor–G protein interactions, second messenger generation (Ca^{2+} and phospholipids), protein synthesis. Available: June 1989. Salary: PGY basis. **Contact: Kenneth M. Baker, M.D., Weis Center for Research, Geisinger Clinic, North Academy Avenue, Danville, PA 17822–2611.** (NW3439)E

STUDENTSHIPS

UNIVERSITY OF CAMBRIDGE
Department of Zoology

RESEARCH STUDENTSHIPS

A number of research studentships will be available in this Department from October 1989. The Research interests of the Department include Molecular Biology, Neurobiology, Developmental Biology, Behaviour Behavioural Ecology, Biomechanics, Ecology, Comparative Physiology and Evolutionary Studies.

Please write for details of these research opportunities to **Mrs J Schreiber, Department of Zoology, Downing Street, Cambridge CB2 3EJ.** (8701)F

UNIVERSITY OF LEICESTER RESEARCH STUDENTSHIPS IN GENETICS AND MOLECULAR BIOLOGY

Studentships are available for training in basic and genetic engineering techniques in a large multi-disciplinary Department. Applications will be considered for the following projects:

1. Bacterial pathogenicity in humans and domestic animals.
2. Bacterial plasmids; their maintenance and conjugative transfer.
3. Cell division mechanisms in *E. coli*; haemolysin secretion and synthesis and assembly of membrane proteins.
4. Differentiation and the mitotic cycle in *Physarum polycephalum*.
5. Function and expression of genes encoded by yeast plasmids; replication mechanisms of yeast plasmids.
6. Function and molecular biology of cytoskeletal roles of novobiocin-binding proteins.
7. Genetic and molecular control of biological and sexual behaviour in *Drosophila*.
8. Genetic variability in small mammal populations; mimicry of ecological processes by examination of effect of artificial selection on *Drosophila* populations.
9. Genetic control and analysis of carbon metabolism in *Aspergillus nidulans*.
10. Highly variable regions of human and animal DNA for use as genetic markers to identify family relationships, analysis of inherited disease and cancer, and use in animal breeding.
11. Protein secretion by Gram negative bacteria; protein engineering of antigens and epitopes.
12. Structure and function of human genes of clinical interest, with particular reference to the collagen family.

Studentships will be funded by SERC and MRC. Applicants should have or expect to obtain a 2.1 or 1st class honours degree in a Biological Science subject preferably including some knowledge of Molecular Genetics or Biochemistry.

Apply by submitting *curriculum vitae* including the names of two academic referees to the **Departmental Secretary, Department of Genetics, University of Leicester, Leicester LE1 7RH, from whom further details may be obtained.** (8755)F

MRC
Medical Research Council

MRC BRAIN METABOLISM UNIT
University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ

RESEARCH STUDENTSHIPS

Applications are invited from final year students expecting to graduate with a first or upper second class honours degree in appropriate branches of science, medicine or veterinary medicine. The MRC Brain Metabolism Unit offers unique training in neuropharmacology and molecular neurobiology. The successful applicants will work on one of the following topics: neuropeptide-receptor interactions and intracellular signalling; molecular and cellular mechanisms in the regulation of neuropeptide and pituitary hormone biosynthesis and secretion; steroid regulation of gene expression. The successful applicants will be registered as research students in the University of Edinburgh. Applications including a full curriculum vitae together with the names of three academic referees should be addressed to the **Administrator, Mrs Anne Milligan, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ,** no later than March 31, 1989. (8638)F

THE UNIVERSITY OF BIRMINGHAM
School of Biochemistry

RESEARCH STUDENTSHIP

funded by the HOME-GROWN CEREALS AUTHORITY

Applications are invited for a three-year PhD research studentship beginning in June, or as arranged, in 1989. The project is to investigate the fundamental biochemical causes of dormacy in barley grains. The successful applicant will join a team engaged in related studies.

Applicants having, or expecting, a I or II degree, which includes Biochemistry or Chemistry, should provide a full *Curriculum Vitae* and the names of two referees. Application forms are available.

Applications and requests for further details, to **Dr D E Briggs, School of Biochemistry, P O Box 363, Birmingham B15 2TT** by 19 April 1989. Telephone: 021-414-5418 quoting reference number 1193.

An Equal Opportunities Employer. (8753)F

MRC LABORATORY OF MOLECULAR BIOLOGY

A number of postgraduate studentships, tenable for up to 3 years, will be available in this laboratory for studies commencing in October 1989. Projects offered are:–

Dr. L. Amos	Studies on proteins involved in intracellular locomotion
Dr. A.C. Bloomer	Crystallographic studies of protein assemblies
Dr. T. Creighton	Experimental studies of protein folding *in vitro* and *in vivo*
Dr. P. Evans	Studies on allostery in phosphofructokinase
Dr. R. Kay	The molecular basis of morphogen action in *Dictyostelium*
Dr. J. Kendrick-Jones	Analysis of myosin structure and function in nonmuscle cells
Dr. J. Kilmartin	Biochemistry and genetics of mitosis in yeast
Dr. A. Leslie	Structural studies of chloramphenicol acetyl transferase
Dr. A. McLachlan	Maximum entropy methods for refining protein structures
Dr. M. Stewart	Directed mutagenesis studies of the molecular basis of interactions in the nuclear envelope
Dr. N. Unwin	3-D structures of membrane channels/ receptors

For further information applicants should write directly to **Dr. A.A. Travers, MRC, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH.** (8758)F

MRC

MEDICAL RESEARCH COUNCIL
Institute of Hearing Research
POSTGRADUATE STUDENTSHIP

The Biology section of the Institute of Hearing Research invites applications for a studentship starting in October 1989 for three years. Two projects are available:

1. Structural and functional development of the inner ear in mice with hereditary deafness, and the role of melanocytes in the development and function of the inner ear (Dr Steel).
2. Single unit responses of the central auditory system to complex sounds such as speech (Dr Palmer).

The institute has good facilities for single unit and gross electrophysiology, light and electron microscopy, excellent computing and workshop support, and in addition has collaborative links with molecular genetics laboratories for genetic studies of deafness.

Applicants should have,or expect to gain, a good degree in a Biological science.

For further details about the projects available, contact **Dr K P Steel, MRC Institute of Hearing Research, University Park, Nottingham NG7 2RD, telephone 0602 223431.** (8732)F

UNIVERSITY COLLEGE LONDON
Department of Physiology
RESEARCH STUDENTSHIPS

Studentships are available for October 89 on projects such as: Long-term potentiation in cerebral cortex, using isolated slices (with Dr L. Bindman).

Reciprocal interactions between pain afferents and inflammation (Dr B. Lynn).

Cell division cycle and second messengers, using techniques in cell physiology and molecular biology (Dr M. Whitaker).

And others in areas of cell and neurophysiology.

Further information from Dr S. Page, Department of Physiology, University College London, Gower St, London WC1E 6BT. (8722)F

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RESEARCH STUDEN
ISOTOPE GEOC

A 3 year studentship, supported by VG Isog the stable isotope geochemistry of fluid pro Fluid inclusion geochemistry will be inv extraction and mass spectrometric techniq The student will be based in the new stable i also have opportunities to utilise research Isogas Ltd. Attendance at international cont The project will be jointly supervised by Dr [(V G Isogas).

Applications, including a brief CV and name **Dr D P Mattey, Department of Geology, RH Hill, Egham, Surrey, TW20 0EX.** To obtain ft Egham (0784) 34455 ext. 3587.

KING'S COLLEGI
University of
DIVISION OF BIOMED
Anatomy and Hun

The Biological Sciences Committee of S research studentship in support of a study and spreading. The project will investigate t as modulators of the adhesion, and subseq cells onto purified glycoproteins of the ext volve a wide range of techniques including glycosylated and deglycosylated fragments, and synthetic peptide sequences which inter cell culture and cell adhesion assays, and c analysis techniques.

Final year students who expect to obtain a honours degree in cell biology, biochemistry are invited to contact: **Dr Gareth Jones, An College London, Strand, London WC2R 2LS.**

UNIVERSITY OF GLASGOW
SERC EARMARKED RESEARCH STUDENTSHIP
PLANT MOLECULAR SCIENCE GROUP

Applications are invited for an SERC earmarked research studentship to study the photoregulation of gene expression in plants. The project will be concerned with the molecular mechanisms linking photoreception to the transcription of specific nuclear genes.

Applicants should hold, or expect to obtain, a First or Upper Second Class Honours Degree in Plant Science, Molecular Biology, Biochemistry, or a related subject. Further details may be obtained from **Dr. G. I. Jenkins, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ**, to whom applications, including a C.V. and the names of two referees should be sent as soon as possible. Candidates may make enquiries by telephoning Dr. Jenkins on 041-339 8855, Extn. 5906. (8749)F

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF PHYSIOLOGY
AND ENVIRONMENTAL SCIENCE
MAFF STUDENTSHIPS

Embryonic antiluteolytic interferons and embryo mortality in cattle

Physiological and biochemical responses to changes in milking frequency and nutrient balance in dairy cows

Applications are invited for two

RESEARCH STUDENTSHIPS

ading to the degree of PhD, tenable from October 1989.

tudy 1 is concerned with the antiluteolytic effect of trophoblastic proteins alpha interferons) associated with the maternal recognition of pregnancy in attle, involving endocrine and receptor binding studies (a co-operative project vith Prof A P F Flint). The successful candidate will be attached to the AFRC esearch Group on Hormones and Farm Animal Reproduction.

tudy 2 is concerned with the relationship between cardiovascular and bio-hemical variables in unrestrained animals during the reproduction/lactation ycle and in response to changes in milking frequency.

Candidates should hold or expect to gain a First or Upper Second Class Honours degree in Biochemistry, Physiology, or in Agricultural Sciences or related disci-pline and, for Study 1, preferably have an interest in molecular biology.

Applicants should write, giving the names and addresses of two academic referees to **Professor G E Lamming (for Study 1) and to Dr T B Mepham (for Study 2) both at Department of Physiology and Environmental Science, University of Nottingham, Faculty of Agricultural & Food Sciences, Sutton Bonington, Loughborough, Leics LE12 5RD.**

Closing date 13th April. (8699)F

UNIVERSITY OF BA

Biochemistry Research S

Research studentships funded by AFRC, S
Bath Bursaries are likely to be available in

1 Study of the origin and specificity o antibodies and their association with
2 The effects of insulin on transl hexose transporters in cultured ad
3 Medical and biotechnological a adhesion
4 Molecular modelling of trypan genase
5 Protein engineering in the study
6 Splicing activities of fungal m design of novel fungicides
7 Molecular Biology of insect ne
8 Glucose metabolism and trans the design of reagents to treat ma

Further information is available fr
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BATH BA2 7AY.

MEDICAL RESE
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Applications

3 YEAR ST

commencing September 198
registered for a postgradu
Glasgow and will work within
ongoing investigation of th
and its relationship to blood
involve the study of the elec
using measurements of ion
together with a variety o
culture and general molecu
Applications to **Dr R Frase**
Infirmary, Dumbarton Roa

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tory neurotransmitters in sens
and feedback mechanisms in v
laboratories, funded by the M
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ance of £1,201.

Applicants should send a full cu
referees to **Professor A. M. S**
whom further details can be o

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NEDO

NEW GRANT FOR INTERNATIONAL JOINT RESEARCH IN MATERIAL FUNCTIONS

New Energy and Industrial Technology Development Organization (NEDO), a semi-governmental agency supervised by the Ministry of International Trade and Industry (MITI), announces the commencement of a new international research grant programme.

The purpose of this programme is to promote creative research on material functions, and at the same time to contribute to the advancement of international exchange.

Research grants will be provided to the "International Joint Research Teams" which fulfil the following conditions.

1. Each team must be composed of four or more researchers.
2. Each team must consist of researchers from two or more different nationalities.
3. The research organizations where the researchers' major activities take place must be located in two or more countries and at least one of the organizations must be in Japan.

This programme supports basic research into material functions in the following areas:

1. Basic research on the elucidation and exploration of material functions.
2. Basic research into the practical use of materials.

Grants will be provided for up to four international research teams and the amount of each grant is up to 30,000,000 yen in FY 1989.

The application deadline is April 28, 1989. Application forms and further details may be obtained from the:

International Joint Research Division
Industrial Technology Department
New Energy and Industrial Technology Development Organization (NEDO)

Address: **29-F, Sunshine Bldg.,**
3-1-1 Higashi-Ikebukuro, Toshima-Ku,
Tokyo, 170, Japan.
Phone No.: 03-987-9357
Telefax No.: 03-981-1536

(W5945)H

STOP PRESS



SANDOZ Foundation for Gerontological Research

Applicants for research grants to the **Sandoz Foundation for Gerontological Research** are reminded that deadlines for applications are:

1st May and **1st November**

Further information and application forms are available from:

Administrative Secretariat, Sandoz Foundation for Gerontological Research, Regional Committee for Europe, P.O. Box 318, Camberley, Surrey GU16 6DN.

(8779)H

SANDOZ

THE CIBA-GEIGY PRIZE FOR RESEARCH IN ANIMAL HEALTH

At the end of 1989, Ciba-Geigy Animal Health will consider submissions for the award of 50,000 Swiss Francs to a scientific worker whose research might be applied to the improvement of animal health through the prevention or treatment of disease.

A submission may be made by the originator of the work or a recommendation may be made by a third party.

The work must be original to the nominee and, while it need not have been carried out during the current year, it should be novel either in concept or development.

The closing date for entries will be 31 May 1989.

Further details and copies of the rules governing the award may be obtained from:

Dr. Theodor Ramp
CIBA-GEIGY Limited
R-1001.B.1.83
CH-4002 Basel
Switzerland

(W5972)N
33 283

King Faisal International Prize

AN INVITATION TO SUBMIT NOMINATIONS FOR THE 1990 KING FAISAL INTERNATIONAL PRIZE IN SCIENCE

The General Secretariat of the King Faisal International Prize has the pleasure in inviting universities, academies, educational institutions and research centers throughout the world to nominate qualified candidates for

the King Faisal International Prize in Science, in the field of

CHEMISTRY

1. A nominee (a living person) must have accomplished an outstanding academic work on the Prize topic; benefiting mankind and enriching human thought in the prize field.
2. The work submitted must be original and published.
3. The work submitted must not have been previously awarded a prize by any international organization.
4. Nominations by individuals or political parties will not be accepted.
5. Nominations should include:
   (a) An official letter by a recognized educational institution, to each nominee, indicating the nominated works.
   (b) Typed C.V. of the nominee's academic background, experience and published works.
   (c) Ten copies of the works submitted for nominations.
   (d) A copy of the nominee's educational certificates.
   (e) Three recent colored photos 10 × 15 cm (4" × 6").
   (f) Full address and telephone number.
6. Nominations will be evaluated by a Selection Committee consisting of highly recognized specialists in the field.
7. More than one person may share the Prize.
8. The winners' names will be announced in January 1990, and the Prize will be awarded in an official ceremony at the headquarter of the King Faisal Foundation in Riyadh, Saudi Arabia, at a later date.
9. The Prize consists of:
   (a) A certificate in the winner's name containing an abstract of the work that qualified him/her for the Prize.
   (b) A gold medal.
   (c) A sum of three hundred and fifty thousand Saudi Riyals, (approximately US$93,333).
10. The latest date for receiving the complete nomination requirements will be (1st of August, 1989).
11. No nomination papers or works will be returned to the senders, whether or not the nominee was awarded the prize.
12. All correspondence must be sent by registered airmail to:

The General Secretariat
King Faisal International Prize
P.O. Box 22476, Riyadh 11495
Kingdom of Saudi Arabia
Telephone: 4652255
Telex 404667 PRIZE SJ
Fax No: 4658685, Cable: JAEZAH

(W5970)N

NATIONAL UNIVERSITY OF SINGAPORE

RESEARCH SCHOLARSHIP

Research Scholarships are available to outstanding university graduates registered for, or admissable to, master's and doctor's degree programmes by research at the National University of Singapore.

The Scholarships are tenable for one year in the first instance and, subject to satisfactory progress renewable annually for another two years.

The value of each Scholarship ranges from S$1000 to S$1500 per month (£1.00 = S$3.50 approximately) and is not subject to income tax; the actual quantum is determined on the candidate's qualifications and experience. No travel allowance is provided.

Research Scholars may be asked to assist in the work of the Departments to which they are attached for not more than six hours a week. They can earn up to S$5400 (gross) per annum at the current rate of remuneration from such departmental work.

The University will assist Research Scholars in obtaining hostel accommodation.

Application forms may be obtained from:

The Registrar,
NATIONAL UNIVERSITY
OF SINGAPORE
10 Kent Ridge Crescent
Singapore 0511
Republic of Singapore

NUS Overseas Office
5 Chesham Street
London SW1, UK

In your request for application forms, please state:-
(a) the field of research you intend to undertake; and
(b) your highest educational qualification.

Applications should be received by the Registrar, National University of Singapore preferably by 31 May 1989. (W5962)H

SEMINARS & SYMPOSIA

Symposium on the Development of the Vascular System 23-26 April '89, Madison, WI.

Topics include: Embryonic vasculogenesis; Endothelial cell differentiation; Cell-matrix interactions; Vascular addressins; Chemotaxis; Angiogenic factors. Speakers: Robert Auerbach, Dianna Ausprunk, Eugene Butcher, Edward Clark, Peter Ekblom, Richard Feinberg, Judah Folkman, Lewis Holmes, David Hootnick, Roger Markwald, Drew Noden, David Packard, Thomas Poole, Werner Risau, Hannu Sariola, Glenn Scherer, David Zaleske, Sally Zigmond. Poster abstracts due April 1st. FAX (608) 262-7319. Registration information: **Center for Dev. Biol., 1117 W Johnson St., Madison, WI53706 (608) 262-1171.** (NW3484)M

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Fifth International Symposium on Cellular Endocrinology

"MOLECULAR CELL BIOLOGY OF LIVER GROWTH AND FUNCTION"

Held in Honor of Dr. Nancy Bucher and Dr. Van Rensselaer Potter

August 13-16, 1989.

Howard Johnson Resort Hotel, Lake Placid, New York.

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risham, "Liver Epithelial Cells and Their Lineages"; **N. Marceau,** "Hepatocyte Progenitors in Hepatocarcinogenesis"; **Guguen-Guillouzo,** "Cell-Cell Interactions and Liver Function"; **S. Thorgeirsson,** "Regulation of Growth, Differentiation Neoplastic Transformation"; **H. Pitot,** "Phenotypic Alterations during Tumor Promotion"

WTH FACTORS AND RECEPTORS

effert, "Mitogens and Hepatocyte Growth Control In Vivo and In Vitro"; **N. Fausto,** "Transforming Growth Factor α and β in Liver eneration"; **G. Michalopoulos,** "Growth Factors and α-1 Adrenergic Receptor in Liver Regeneration and Carcinogenesis" **B. Carr,** e and Expression of Transforming Growth Factor ß"; **W. Russell,** "TGF-ß in Liver Regeneration"; **W. L. McKeehan,** parin-Binding Growth Factors in Liver"; **T. Nakamura,** "Hepatocyte Growth Factor (HGF) and Liver Regeneration"; **J. D. Yager,** "Liver Growth Stimulation by Tumor Promoter Ethinyl Estradiol"

RNA, PROTEIN SYNTHESIS

um, "Regenerating Liver and Eukaryotic DNA Replication"; **L. Fessler;** "Molecular Structure and Function of Basement anes"; **L.Reid,** "Proteoglycan Regulation of Liver Gene Expression". **S. Farmer,** "Cell-Matrix Interaction and Gene sion"; **R. Costa,** "Transcriptional Regulatory Proteins and Liver-Specific Genes"; **H. Baumann,** "Acute Phase Plasma Protein es"; **G. Blobel,** "Intracellular Protein Traffic".

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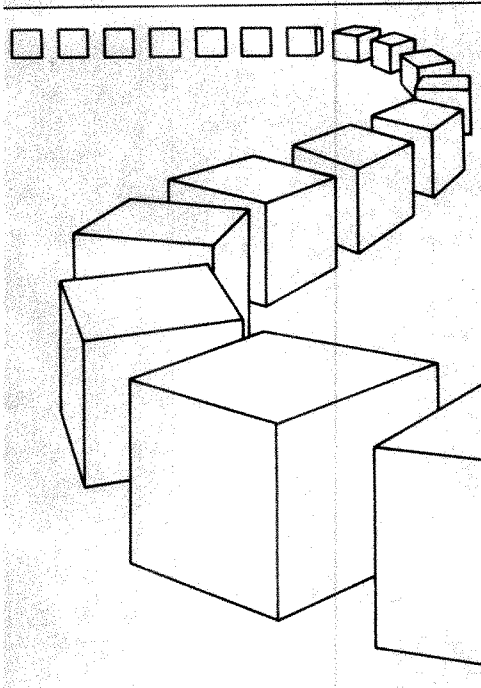
som, "Gene Expression in SV40-Transformed Hepatocytes"; **D. Paul,** "Growth Regulation in Transgenic Hepatocytes". **Anderson,** "Retroviral Gene Transfer"; **S. Woo,** "Retroviral Gene Transfer in Hepatocytes"; **J. Wilson,** "Correction of Hepatic -Receptor Deficiency by Gene Transfer"; **G. Wu,** "Receptor-Mediated Delivery of Genes to Hepatocytes"; **J. Roy-Chowdhury,** er Cell Transplantation for Liver Disease".

POSTER SUBMISSIONS ARE INVITED. FOR MORE INFORMATION CONTACT:

THE ORGANIZING COMMITTEE.

W. Alton Jones Cell Science Center, Inc.,
10 Old Barn Road, Lake Placid, New York, 12946.
or CALL (518) 523-1287

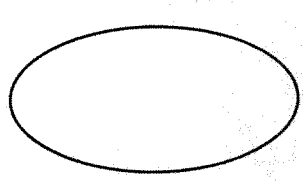
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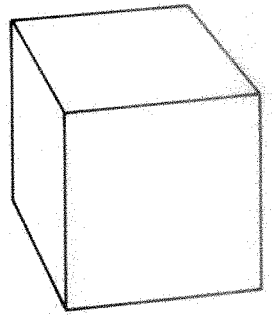


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DNA RELATED METHODS IN HUMAN GENETICS:

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Division of Biochemistry, United Medical and Dental Schools of Guy's and St. Thomas's Hospitals, Guy's Campus, London.

An intensive laboratory based course to introduce participants to modern methods of DNA analysis.

Programme:

1. Pulse-field gel electrophoresis (PFGE) – DNA digests, blotting, preparation and labelling of probes.
2. Mapping by PFG.
3. Analysis of large DNA fragments cloned in yeast artificial chromosomes (YACS).
4. Gene amplification by the polymerase chain reaction.

Informal tutorials and evening seminars (by invited speakers) will supplement the laboratory sessions.

Course Directors:
KAY E. DAVIES, ANNA-MARIA FRISCHAUF and WILLIAM BROWN.

Invited Speakers:
DUNCAN CAMPBELL, NICK HASTIE, MARTIN HOOPER, ED SOUTHERN, PAUL TRAVERS.

Participants:
Applicants should be post-doctoral scientists (or close to) engaged in clinical, veterinary or basic biomedical research. All participants will be expected to have a basic competence in recombinant DNA techniques.

Applications:
There are no formal application forms, but applicants should send a copy of their full **curriculum vitae** together with a 300 word outline of their research plans to:-
Dr. Pelin Faik, Course Coordinator, Division of Biochemistry, UMDS, Guy's Campus, London Bridge, London SE1 9RT. (Tel: 01-403 6998) (FAX: 01-407 5281)
Closing date for applications is April 30th.
This is a re-advertisement since it has proved possible to increase the number of places available. Previous applicants should **not** re-apply. Further information may be obtained by contacting Dr. Faik.

(8784)C

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EMBO COURSE

CRYO SECTION IMMUNOLABELLING AND QUANTITATION

EMBL, HEIDELBERG AUG 14–24, 1989

This course will consist of two parts. *Part A:* 6 days of practical and theoretical details of cryo-sectioning for immunocytochemistry, primarily for electron microscopy. Teachers will include: J. Dubochet, G. Griffiths, Lucocq, J.W. Slot and K. Tokuyasu. *Part B:* 3–4 days theory and practice tereology for biology. Teaching staff: L. Cruz-Orive, H.J. Gunderson, oeler and T. Mattfield. At the end of this part the aim is to integrate of stereology for quantitation in immunocytochemistry.

e is primarily aimed at Ph.D. students as well as more experi- ntists. Applicants should send a brief CV which should include a of their research interests. Preference will be given to those ho need to use both immunocytochemistry and quantitative s in their research.

fore May 15 to: **G. Griffiths, EMBL, Postfach 10.2209, g, FRG.**

(8730)C

Prediction of Percutaneous Penetration
Methods Measurements Modelling
International Conference
4-6 April 1989, UMIST

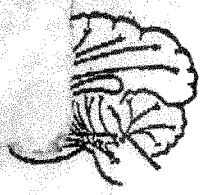
Topics include: In vivo methodology; In Vitro methodology, Formulation effects; Animal models; Mechanisms and enhancement; Prediction and modelling; Regulatory affairs.

Invited speakers: R Marks (UK), J B Knaak (USA), A Rougier (France); R L Bronaugh (USA), R E Grissom (USA); G Ridout (UK), H E Bod (Netherlands), J C Jamoulle (France), K A Walters (UK), R H G (USA), H M Wolff (FRG), R C Scott (UK), B W Barry (UK), H Schae (FRG), M S Roberts (NZ), D E Leahy (UK), J Hadgraft (UK), J N McDougal (USA), A D Martin (UK), P H Dugard (UK), T Farber (USA), C C Peck (USA).

Programme will include invited presentations, selected submitted papers, workshops and poster sessions.

Registration information: Christine Smith, PPP Conference, Richmond Bridge House, 417-421 Richmond Road, Twickenham, Middx, TW1 2EX Tel: 01-892 3637 Fax: 01-744 2142.

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CORRESPONDENCE
M. Yoshida (SECRETARY)
Department of Neurology
Jichi Medical School
Minamikawachi, Tochigi
329-04 JAPAN

PLENARY LECTURE 1
Professor Masao Ito
"Brain mechanisms underlying specific symptoms of Alzheimer's and Parkinson's Diseases"

PLENARY LECTURE 2
Professor Peter Davies
"The Neurobiology of Alzheimer's Disease"

PLENARY LECTURE 3
Professor Arvid Carlsson
"Role of Brain Monoamines in Normal and Pathological Aging"

ORAL PRESENTATIONS
(invited speakers)
(20 or 30 minutes each)
50 papers from abroad
20 papers from Japan

FREE COMMUNICATIONS
(poster presentations)
more than 100 papers are expected

SCIENTIFIC PROGRAM
The scientific program of the Conference will include invited oral lectures, round table discussion and poster presentation. The tentative key topics are:

1. Etiology and potential tool of breakdown of the neuronal system.
2. Biological markers of Alzheimer's and Parkinson's diseases.
3. Experimental model of Alzheimer's and Parkinson's diseases.
4. Clinical, pathological, biological and therapeutic aspects of Alzheimer's and Parkinson's disease.
5. Drug development for Alzheimer's and Parkinson's diseases.
6. Future strategies in Alzheimer's and Parkinson's diseases.

(W5961)C

COURSES continued on page 42

CONGRESS

ıe International Association of Biological Standardization will hold its 21st Congress at Annecy, France on October 4-6, 1989, with the title:

"PROGRESS IN ANIMAL RETROVIRUSES."

ıe subject will be exclusively devoted to the:
retrovirus status of cells and animals used for assays,
evaluation of detection methods,
mmunization and biotherapy approaches, and
elevance to human retroviruses infections.

r further information, you may contact:

Dr. Daniel Gaudry
Secretary of the 21st Congress of the IABS
254, rue M. Merieux
69007 LYON – FRANCE
Tel: 33.72.72.30.25
Fax: 33.72.72.30.68

(W5965)Q

LECTURES



ROYAL SOCIETY DISCUSSION MEETINGS
MAY 1989

24 and 25 May
'THE FAST-NEUTRON-BREEDER FISSION REACTOR'

Organized by Sir Peter Hirsch, F.R.S., Dr T.N. Marsham, F.R.S., Dr R.S. Pease, F.R.S. and Dr B.L. Eyre.
Speakers: R.S. Pease, F.R.S., G. Vendryes, M. Koehler, M.F. Troyanov, J.D. Griffith, S. Sawai, A.M. Broomfield, R. Lallement, R.H. Allard, L.E.J. Roberts, F.R.S., H-H. Hennies, B.L. Eyre, M. Rapin, T.N. Marsham, F.R.S. and J.G. Collier.

The Discussion Meeting on 25 May will be followed by the Lecture at 17.00 given by the Rt. Hon. the Lord Perry of F.R.S. The title of his lecture is **'Science and education'**

Registration details (no fee for attendance): **Miss C.A (ref. DMN/CAJ), The Royal Society, 6 Carlton House London SW1Y 5AG. (Tel: 01-839 5561, ext. 278. Tel**



E N I I : European Network of Immunology Institutes

The fourth European Network of Immunology Institutes Conference, chaired by D. Mathis a R. McDonald, will be held on the island of Les Embiez, France from May 16th-20th.

There will be seven plenary sessions on: B cell differentiation, Antibody structure and engineerir T cell differentiation, The (antigen-MHC-T cell receptor) complex, Hematopoietic stem cel early lymphoid precursors, Molecular aspects of autoimmunity, Inherited immune disorders.

The 1st Neils Jërne lecture will be given by Dr. B. A. Askonas (London/Oxford).

Influenza Virus, a Challenge to the Immunologist.

Speakers will include: K. Rajewsky, M. Reth, C. Goodnow, M. Harnett, F. Kroese, R. Poljak, S. War A. Rees, J. Anglister, H von Boehmer, U. Staerz, J. Allison, A. Lanzavecchia, S. Buus, C. Benois M. Davis, B. Devaux, N. Le Douarin, I. Weissman, P. Kincade, J. Visser, J. Gallagher, H. Wekerle, H Acha-Orbea, J. Epplen, R. Kofler, I. Förster, B. Jordan, C. Terhorst, R. Levinsky, A. Fischer, W. Reiti

Participation limited to 150. Time will be given for registered participants to present data as te minute papers or in a poster session.

Students and postdoctoral fellows are encouraged to apply. A limited number of bursaries will be available.

Applications should include a 200 word abstract and a 1 page C.V., as well as telephone and telefax numbers, to:

1989 ENII Conference, c/o Dr. Diane Mathis, INSERM U. 184/LGME, 11, rue Humann, 67085 STRASBOURG CEDEX FRANCE

The registration fee, which includes transport from Marseille Airport and full board, will be 2,300 FF.

(W5964)C

Technology Management Group



DRUG DELIVERY OF PROTEINS

June 12, 13, and 14, 1989,
Boston, Massachusetts, U.S.A.

TMG announces a conference on the key enabling technologies for biotechnology therapeutics for the 1990's

Planned speakers include key individuals from:

Alza	Elan	Osmed
California Biotechnology	The Liposome Company	Syntex
Cetus	Liposome Technology	University of Southern California
Ciba-Geigy	Nova Pharmaceuticals	Technology Management Group

Planned presentations include:

Controlled release of peptides
Applications of polymers for delivery of growth factors and other peptides
Delivery of growth factors for wound healing
Delivery of interferons
Delivery of interleukin-2
Worldwide market opportunities and activities of companies
Biodegradable polymer matrix for growth factor delivery to hard tissue
Monoclonal antibody-based delivery
Liposomes for peptide delivery
Nasal administration of hormones, including clinical results
Non-invasive mucosal delivery of growth factors, hormones and other peptides
Transdermal delivery of peptides
Red blood cells as a delivery vehicle
Lipid-based delivery of growth hormones and other peptides
Site-specific targetting of therapeutic proteins

To reserve a place, or for more information, contact:

Technology Management Group
25 Science Park, New Haven, Connecticut 06511 U.S.A.
Tel: (203) 786-5445, Fax: (203) 786-5449 (NW3452)C

CONFERENCE

The Second International Workshop on Cytokines
10 to 14 December 1989
Hilton Head, South Carolina

GOALS: a) to encompass and integrate the most recei data concerning cytokine synthesis, release, regulatio actions, and interactions, and b) to assess the in viv effects of these cytokines on cell, tissue, organ, and o ganism metabolism, physiology, and immunity. TOPICS structure, gene expression, regulation of synthesis an release, receptors and post receptor events, cytokir activities, alone and in combination, assays for and inhib tors of the cytokines. CYTOKINES TO BE CONSIDERED the interleukins, the interferons, TNF, TGF, PDGF, chem tactic factors, the various colony stimulating factors, bor and connective tissue growth factors, as well as new identified activities/factors.

INVITED SPEAKERS: Robert Friedman (interferons Tadamitsu Kishimoto (IL-6), Thomas Maciag (ECGF Lloyd Old (TNF), Russell Ross (PDGF), Ruth Sager (GRO Arthur Silverstein (historical perspective). The content c the rest of the meeting will be based on submitted a stracts.

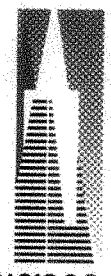
Abstracts will be published in the premiere issue CYTOKINE (W B Saunders Company). Deadline for recei of abstracts is 15 June 1989. For abstract forms, meetin information, and registration forms contact: **Sherwoo M. Reichard, Cytokines, c/o RES Society, Box 3044 MCC Augusta, GA 30912 USA.** (NW3168)C

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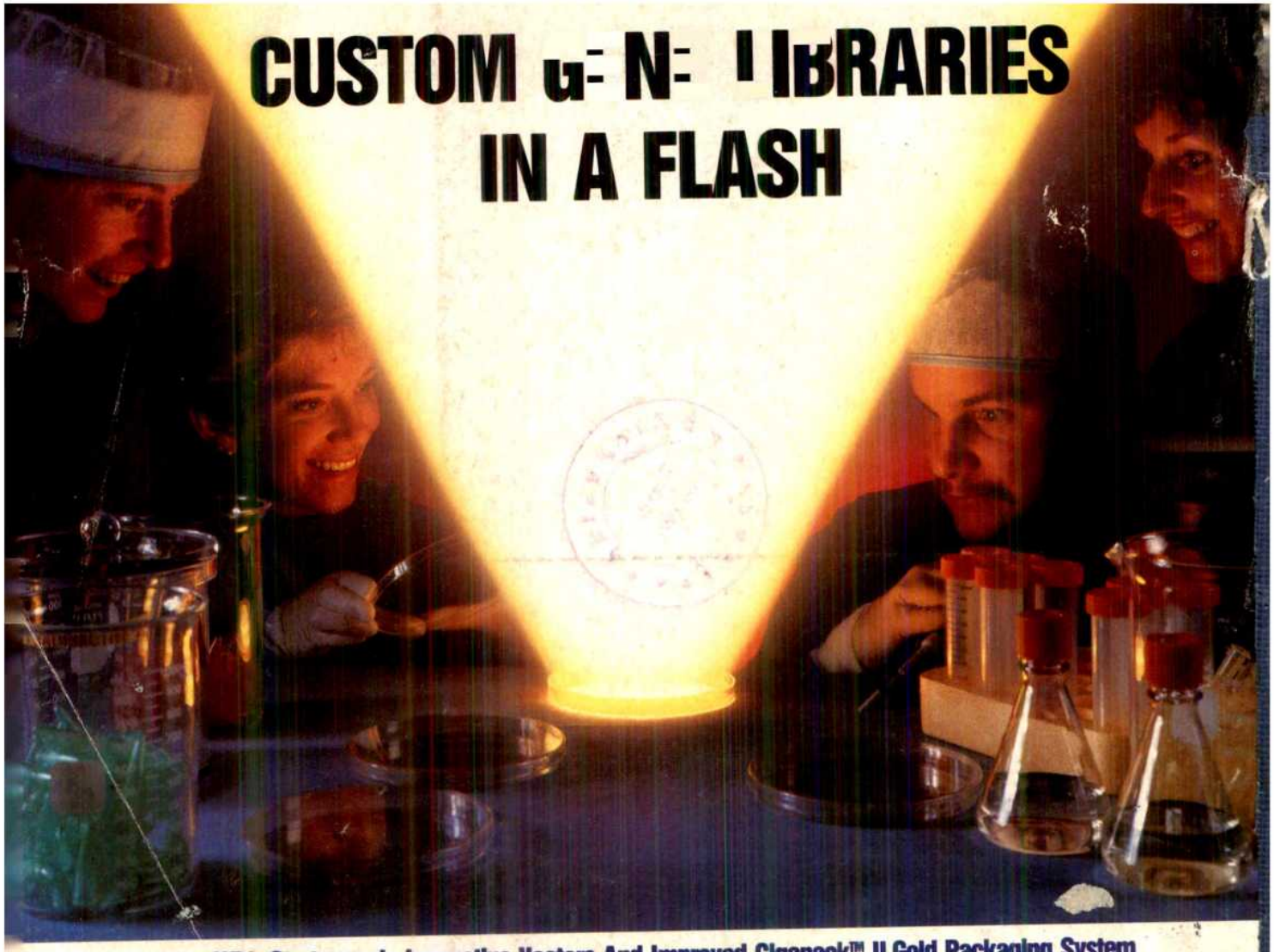
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